NIL

EO/US
PCT/AU00/00436

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark Office, PCT
2011 South Clark Place Room CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing: 23 November 2000 (23.11.00)	
International application No.: PCT/AU00/00436	Applicant's or agent's file reference: 2290375/MRO
International filing date: 11 May 2000 (11.05.00)	Priority date: 13 May 1999 (13.05.99)
Applicant: ANKENBAUER, Robert, Gerard et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International preliminary Examining Authority on:

11 September 2000 (11.09.00)

[ ] in a notice effecting later election filed with the International Bureau on:

_____

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No.: (41-22) 740.14.35	Authorized officer: J. Zahra Telephone No.: (41-22) 338.83.38

Form PCT/IB/331 (July 1992)

3660376

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 2290375	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416).
International application No. **PCT/AU00/00436**	International filing date *(day/month/year)* 11 May 2000	Priority Date *(day/month/year)* 13 May 1999

International Patent Classification (IPC) or national classification and IPC

Int. Cl. [7] C07K 14/195, C12N 15/31, A61K 39/02, A61P 1/00, C07K 16/12, G01N 33/537, C12Q 1/04

Applicant

PIG RESEARCH AND DEVELOPMENT CORPORATION et al

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 3 sheets, including this cover sheet.

☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of sheet(s).

3. This report contains indications relating to the following items:

I	☒	Basis of the report
II	☐	Priority
III	☐	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☐	Lack of unity of invention
V	☒	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☐	Certain documents cited
VII	☐	Certain defects in the international application
VIII	☐	Certain observations on the international application

Date of submission of the demand 11 September 2000	Date of completion of the report 26 September 2000
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized Officer **K. LEVER** Telephone No. (02) 6283 2254

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/AU00/00436

I. Basis of the report

1. With regard to the **elements** of the international application:*

[X] the international application as originally filed.

[ ] the description, pages , as originally filed,
pages , filed with the demand,
pages , received on with the letter of

[ ] the claims, pages , as originally filed,
pages , as amended (together with any statement) under Article 19,
pages , filed with the demand,
pages , received on with the letter of

[ ] the drawings, pages , as originally filed,
pages , filed with the demand,
pages , received on with the letter of

[ ] the sequence listing part of the description:
pages , as originally filed
pages , filed with the demand
pages , received on with the letter of

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language which is:

[ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

[ ] the language of publication of the international application (under Rule 48.3(b)).

[ ] the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, was on the basis of the sequence listing:

[ ] contained in the international application in written form.

[X] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readable form.

[ ] The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

4. [ ] The amendments have resulted in the cancellation of:

[ ] the description, pages

[ ] the claims, Nos.

[ ] the drawings, sheets/fig.

5. [ ] This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report*

INTERNATIONAL PRELIMIN[redacted] EXAMINATION REPORT

[redacted]national application No.
PCT/AU00/00436

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-48	YES
	Claims		NO
Inventive step (IS)	Claims	1-48	YES
	Claims		NO
Industrial applicability (IA)	Claims	1-48	YES
	Claims		NO

2. Citations and explanations (Rule 70.7)

Citations:

D1: Veterinary Pathology (March 1998) 35 (2) 153-6. Boye M et al. 'Specific Detection of *Lawsonia intracellularis* in Porcine Proliferative Enteropathy Inferred from Fluorescent rRNA In Situ Hybridization'.

D2: WO 9639629 A1 (NOBL LABORATORIES INC) 12 December 1996.

D3: WO 9720050 A1 (PIG RESEARCH & DEVELOPMENT CORP ET AL) 5 June 1997.

Explanations:

The present application is directed to an isolated or recombinant immunogenic polypeptide, which comprises, mimics or cross-reacts with a B-cell or T-cell epitope of the SodC polypeptide derived from Lawsonia spp. The above-cited documents all refer to Lawsonia spp but do not disclose or suggest the SodC polypeptide nor do they disclose Sequence 1 of the current application.

Therefore it is considered that Claims 1-48 are Novel, Inventive and demonstrate Industrial Applicability.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION**	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
2290375/MRO		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/AU00/00436	11 May 2000	13 May 1999

Applicant

PIG RESEARCH AND DEVELOPMENT CORPORATION et al

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

[ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing:

[ ] contained in the international application in written form.

[X] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readable form.

[ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (See Box II).

4. With regard to the **title,**

[ ] the text is approved as submitted by the applicant.

[X] the text has been established by this Authority to read as follows:

***Lawsonia* derived gene and related SodC polypeptides, peptides and proteins and their uses**

5. With regard to the **abstract,**

[X] the text is approved as submitted by the applicant

[ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No.

[ ] as suggested by the applicant.

[ ] because the applicant failed to suggest a figure

[ ] because this figure better characterizes the invention

[X] None of the figures

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU00/00436

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl. [7]: C07K 14/195, C07H 21/04, A61K 39/02, A61P 1/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
STN:WIPS-Lawsonia, MEDLINE, HCA, BIOSIS- Lawsonia, polypeptide, gene, protein, peptide, immunogen, recombin ANGIS: sequence search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Veterinary Pathology (March 1998) 35 (2) 153-6. Boye M et al. 'Specific Detection of *Lawsonia intracellularis* in Porcine Proliferative Enteropathy Inferred from Fluorescent rRNA In Situ Hybridization'. See whole document.	37-48
A	WO 9639629 A1 (NOBL LABORATORIES INC) 12 December 1996. See whole document	1-48
A	WO 9720050 A1 (PIG RESEARCH & DEVELOPMENT CORP ET AL) 5 June 1997. See whole document.	1-48

☐ Further documents are listed in the continuation of Box C ☒ See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 June 2000	05 JUL 2000
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized officer K. LEVER Telephone No : (02) 6283 2254

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/AU00/00436

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
WO	9639629	AU	62627/96	CA	2222643	EP	843818
		US	5885823	US	5714375		
WO	9720050	AU	76141/96	CA	2236574	EP	871735

END OF ANNEX

PCT REQUEST

2290375/MRO

Original (for **SUBMISSION**) - printed on 11.05.2000 01:19:38 PM

0	**For receiving Office use only**	
0-1	International Application No.	
0-2	International Filing Date	
0-3	Name of receiving Office and "PCT International Application"	
0-4	**Form - PCT/RO/101 PCT Request**	
0-4-1	Prepared using	PCT-EASY Version 2.90 (updated 08.03.2000)
0-5	**Petition** The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty	
0-6	**Receiving Office (specified by the applicant)**	Australian Patent Office (RO/AU)
0-7	**Applicant's or agent's file reference**	2290375/MRO
I	**Title of invention**	NOVEL LAWSONIA SSP. GENE AND USES THEREFOR V
II	**Applicant**	
II-1	This person is:	applicant only
II-2	Applicant for	all designated States except US
II-4	Name	PFIZER PRODUCTS INC
II-5	Address:	Eastern Point Road Groton, CT 06340 United States of America
II-6	State of nationality	US
II-7	State of residence	US
III-1	**Applicant and/or inventor**	
III-1-1	This person is:	applicant only
III-1-2	Applicant for	all designated States except US
III-1-4	Name	PIG RESEARCH AND DEVELOPMENT CORPORATION
III-1-5	Address:	3rd Floor Industry House 10 National Circuit Barton, Australian Capital Territory 2600 Australia
III-1-6	State of nationality	AU
III-1-7	State of residence	AU

PCT REQUEST 2290375/MRO

Original (for **SUBMISSION**) - printed on 11.05.2000 01:19:38 PM

III-2	**Applicant and/or inventor**	
III-2-1	This person is:	applicant only
III-2-2	Applicant for	all designated States except US
III-2-4	Name	AGRICULTURE VICTORIA SERVICES PTY LTD
III-2-5	Address:	475 Mickleham Road Attwood, Victoria 3049 Australia
III-2-6	State of nationality	AU
III-2-7	State of residence	AU
III-3	**Applicant and/or inventor**	
III-3-1	This person is:	applicant and inventor
III-3-2	Applicant for	US only
III-3-4	Name (LAST, First)	ANKENBAUER, Robert Gerard
III-3-5	Address:	104 Castle Hill Road Pawcatuck, CT 06379 United States of America
III-3-6	State of nationality	US
III-3-7	State of residence	US
III-4	**Applicant and/or inventor**	
III-4-1	This person is:	applicant and inventor
III-4-2	Applicant for	US only
III-4-4	Name (LAST, First)	HASSE, Detlef
III-4-5	Address:	4 Scullin Court Sunbury, Victoria 3429 Australia
III-4-6	State of nationality	AU
III-4-7	State of residence	AU
III-5	**Applicant and/or inventor**	
III-5-1	This person is:	applicant and inventor
III-5-2	Applicant for	US only
III-5-4	Name (LAST, First)	PANACCIO, Michael
III-5-5	Address:	112 Hill Road North Balwyn, Victoria 3104 Australia
III-5-6	State of nationality	AU
III-5-7	State of residence	AU
III-6	**Applicant and/or inventor**	
III-6-1	This person is:	applicant and inventor
III-6-2	Applicant for	US only
III-6-4	Name (LAST, First)	ROSEY, Everett Lee
III-6-5	Address:	457 Route 164 Preston, CT 06365 United States of America
III-6-6	State of nationality	US
III-6-7	State of residence	US

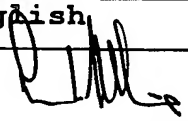
PCT REQUEST 2290375/MRO

Original (for **SUBMISSION**) - printed on 11.05.2000 01:19:38 PM

III-7	**Applicant and/or inventor**	
III-7-1	This person is:	applicant and inventor
III-7-2	Applicant for	US only
III-7-4	Name (LAST, First)	WRIGHT, Catherine
III-7-5	Address:	49/422 Cardigan Street Carlton, Victoria 3053 Australia
III-7-6	State of nationality	AU
III-7-7	State of residence	AU
IV-1	**Agent or common representative; or address for correspondence** The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as:	agent
IV-1-1	Name (LAST, First)	OLIVE, Mark, R
IV-1-2	Address:	DAVIES COLLISON CAVE 1 LITTLE COLLINS STREET MELBOURNE, Victoria 3000 Australia
IV-1-3	Telephone No.	+61 3 9254 2777
IV-1-4	Facsimile No.	+61 3 9254 2770
IV-2	**Additional agent(s)**	additional agent(s) with same address as first named agent
IV-2-1	Name(s)	SLATTERY, John, M; CAINE, Michael, J
V	**Designation of States**	
V-1	Regional Patent (other kinds of protection or treatment, if any, are specified between parentheses after the designation(s) concerned)	AP: GH GM KE LS MW SD SL SZ TZ UG ZW and any other State which is a Contracting State of the Harare Protocol and of the PCT EA: AM AZ BY KG KZ MD RU TJ TM and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT EP: AT BE CH&LI CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE and any other State which is a Contracting State of the European Patent Convention and of the PCT OA: BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG and any other State which is a member State of OAPI and a Contracting State of the PCT
V-2	National Patent (other kinds of protection or treatment, if any, are specified between parentheses after the designation(s) concerned)	AE AG AL AM AT AU AZ BA BB BG BR BY CA CH&LI CN CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW

PCT REQUEST 2290375/MRO

Original (for **SUBMISSION**) - printed on 11.05.2000 01:19:38 PM

V-5	**Precautionary Designation Statement** In addition to the designations made under items V-1, V-2 and V-3, the applicant also makes under Rule 4.9(b) all designations which would be permitted under the PCT except any designation(s) of the State(s) indicated under item V-6 below. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit.		
V-6	**Exclusion(s) from precautionary designations**	NONE	
VI-1	**Priority claim of earlier national application**		
VI-1-1	Filing date	13 May 1999 (13.05.1999)	
VI-1-2	Number	60/133,989	
VI-1-3	Country	US	
VII-1	**International Searching Authority Chosen**	Australian Patent Office (ISA/AU)	
VIII	**Check list**	number of sheets	electronic file(s) attached
VIII-1	Request	5	-
VIII-2	Description (excluding sequence listing part)	62	-
VIII-3	Claims	8	-
VIII-4	Abstract	1	2290375.txt
VIII-5	Drawings	1	-
VIII-6	Sequence listing part of description	4	-
VIII-7	TOTAL	81	
	Accompanying items	paper document(s) attached	electronic file(s) attached
VIII-8	Fee calculation sheet	✓	-
VIII-9	Separate signed power of attorney	✓	-
VIII-15	Nucleotide and/or amino acid sequence listing in computer readable form		separate diskette
VIII-16	PCT-EASY diskette	-	diskette
VIII-18	**Figure of the drawings which should accompany the abstract**		
VIII-19	**Language of filing of the international application**	English	
IX-1	**Signature of applicant or agent**		
IX-1-1	Name (LAST, First)	OLIVE, Mark, R	

FOR RECEIVING OFFICE USE ONLY

10-1	**Date of actual receipt of the purported international application**	

PCT REQUEST

2290375/MRO

Original (for **SUBMISSION**) - printed on 11.05.2000 01:19:38 PM

10-2 10-2-1 10-2-2	**Drawings:** Received Not received	
10-3	**Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application**	
10-4	**Date of timely receipt of the required corrections under PCT Article 11(2)**	
10-5	**International Searching Authority**	**ISA/AU**
10-6	**Transmittal of search copy delayed until search fee is paid**	

FOR INTERNATIONAL BUREAU USE ONLY

11-1	**Date of receipt of the record copy by the International Bureau**	

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To: Agent :

DAVIES COLLISON CAVE
1 Little Collins Street
MELBOURNE VIC 3000

PCT

NOTIFICATION OF RECEIPT
OF DEMAND BY COMPETENT INTERNATIONAL
PRELIMINARY EXAMINING AUTHORITY

(PCT Rule 59.3(e) and 61.1(b), first sentence
and Administrative Instructions, Section 601(a))

Date of mailing *(day/month/year)*	18 SEP 2000 (18/9/00)
Applicant's or agent's file reference: 2290375	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/AU00/00436	11 MAY 2000 (11/5/00)	13 MAY 1999 (13/5/99)

Applicant: Pfizer Products Inc. (et al.)

1. The applicant is hereby **notified** that this International Preliminary Examining Authority considers the following date as the date of receipt of the demand for international preliminary examination of the international application:

   11 SEP 2000 (11/9/00)

2. That date of receipt is:

   [X] the actual date of receipt of the demand by this Authority (Rule 61.1(b)).

   [ ] the actual date of receipt of the demand on behalf of this Authority (Rule 59.3(e)).

   [ ] the date on which this Authority has, in response to the Invitation to correct defects in the demand (Form PCT/IPEA/404), received the required corrections.

3. [ ] **Attention:** That date of receipt is **AFTER** the expiration of 19 months from the priority date. Consequently, the elections(s) made in the demand does (do) not have the effect of postponing the entry into the national phase until 30 months from the priority date (or later in some Offices)(Article 39(1)). Therefore, the acts for entry into the national phase must be performed within 20 months from the priority date (or later in some Offices) (Article 22). For details, see the *PCT Applicant's Guide, Volume II.*

   [ ] *(If applicable)* This notification confirms the information given by telephone, facsimile transmission or in person on:

   _____

4. Only where paragraph 3 applies, a copy of this notification has been sent to the International Bureau.

Name and mailing address of the IPEA/AU	Authorized officer
AUSTRALIAN PATENT OFFICE **PO BOX 200, WODEN ACT 2606, AUSTRALIA** E-mail: pct@ipaustralia.gov.au Facsimile No. 02 6285 3929	JOSEPH BRESIC Telephone No. 02 6283 2357

Form PCT/IPEA/402 (July 1998)

The demand must be filed directly with the competent International Preliminary Examining Authority or, if two or more Authorities are competent, with the one chosen by the applicant. The full name or two-letter code of that Authority may be indicated by the applicant on the line below.

IPEA/_____

PCT

CHAPTER II

DEMAND

under Article 31 of the Patent Cooperation Treaty:
The undersigned requests that the international application specified below be the subject of international preliminary examination according to the Patent Cooperation Treaty and hereby elects all eligible States (except where otherwise indicated).

For International Preliminary Examining Authority use only

Identification of IPEA	Date of receipt of DEMAND

Box No. I IDENTIFICATION OF THE INTERNATIONAL APPLICATION		Applicant's or agent's file reference 2290375/MRO
International application No. PCT/AU00/00436	International filing date (*day/month/year*) 11 May, 2000 (11/5/00)	(Earliest) Priority date (*day/month/year*) 13 May, 1999 (13/5/99)

Title of invention
Lawsonia derived gene and related SodC polypeptides, peptides and proteins and their uses

Box No. II APPLICANT(S)

Name and address: *(Family name followed by given name: for a legal entity, full official designation. The address must include postal code and name of country.)*

PFIZER PRODUCTS INC.
Eastern Point Road
Groton, CT 06340
United States of America

Telephone No.:
Facsimile No.:
Teleprinter No.:

State *(that is, country)* of nationality:	State *(that is, country)* of residence:
UNITED STATES OF AMERICA	UNITED STATES OF AMERICA

Name and address: *(Family name followed by given name: for a legal entity, full official designation. The address must include postal code and name of country.)*

PIG RESEARCH AND DEVELOPMENT CORPORATION
3rd Floor, Industry House
10 National Circuit
Barton Australian Capital Territory 2600
Australia

State *(that is, country)* of nationality:	State *(that is, country)* of residence:
AUSTRALIA	AUSTRALIA

Name and address: *(Family name followed by given name: for a legal entity, full official designation. The address must include postal code and name of country.)*

AGRICULTURE VICTORIA SERVICES PTY. LTD.
475 Mickleham Road
Attwood
Victoria 3049
Australia

State *(that is, country)* of nationality:	State *(that is, country)* of residence:
AUSTRALIA	AUSTRALIA

[X] Further applicants are indicated on a continuation sheet.

International application no.
PCT/AU00/00436

Continuation of Box No. II APPLICANT(S)

If none of the following sub-boxes is used, this sheet should not be included in the demand.

Name and address: *(Family name followed by given name: for a legal entity, full official designation. The address must include postal code and name of country.)*

ANKENBAUER, Robert Gerard
104 Castle Hill Road
Pawcatuck, CT 06379
United States of America

State *(that is, country)* of nationality:	State *(that is, country)* of residence:
UNITED STATES OF AMERICA	UNITED STATES OF AMERICA

Name and address: *(Family name followed by given name: for a legal entity, full official designation. The address must include postal code and name of country.)*

HASSE, Detlef
4 Scullin Court
Sunbury, Victoria 3429
Australia

State *(that is, country)* of nationality:	State *(that is, country)* of residence:
AUSTRALIA	AUSTRALIA

Name and address: *(Family name followed by given name: for a legal entity, full official designation. The address must include postal code and name of country.)*

PANACCIO, Michael
112 Hill Road
North Balwyn
Victoria 3104
Australia

State *(that is, country)* of nationality:	State *(that is, country)* of residence:
AUSTRALIA	AUSTRALIA

Name and address: *(Family name followed by given name: for a legal entity, full official designation. The address must include postal code and name of country.)*

ROSEY, Everett Lee
457 Route 164
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[X] Further applicants are indicated on another continuation sheet.

International application no.
PCT/AU00/00436

Continuation of Box No. II APPLICANT(S)

If none of the following sub-boxes is used, this sheet should not be included in the demand.

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International application no.
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The following person is [X] agent [ ] common representative

and [X] has been appointed earlier and represents the applicant(s) also for international preliminary examination.

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[ ] is hereby appointed, specifically for the procedure before the International Preliminary Examining Authority, in addition to the agent(s)/common representative appointed earlier.

Name and address: *(Family name followed by given name: for a legal entity, full official designation. The address must include postal code and name of country.)*

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Statement concerning amendments:*

1. The applicant wishes the international preliminary examination **to start on the basis of:**

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[ ] as amended under Article 34

the claims [X] as originally filed
[ ] as amended under Article 19 (together with any accompanying statement)
[ ] as amended under Article 34

the drawings [X] as originally filed
[ ] as amended under Article 34

2. [ ] The applicant wishes any amendment to the claims under Article 19 to be considered as reversed.

3. [ ] The applicant wishes the start of the international preliminary examination **to be postponed** until the expiration of 20 months from the priority date unless the International Preliminary Examining Authority receives a copy of any amendments made under Article 19 or a notice from the applicant that he does not wish to make such amendments (Rule 69. 1(d)). *This check-box may be marked only where the time limit under Article 19 has not yet expired.)*

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The applicant hereby **elects all eligible States** ***(that is, all States which have been designated and which are bound by Chapter II of the PCT)***

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International application no.
PCT/AU00/00436

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The demand is accompanied by the following elements, in the language referred to in Box No. IV, for the purposes of international preliminary examination:

				For International Preliminary Examining Authority use only	
				received	not received
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3.	copy (or, where required, translation) of amendments under Article 19	:	sheets	☐	☐
4.	copy (or, where required, translation) of statement under Article 19	:	sheets	☐	☐
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The demand is also accompanied by the item(s) marked below:

1. ☐ fee calculation sheet
2. ☐ separate signed power of attorney
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5. ☐ nucleotide and or amino acid sequence listing in computer readable form
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Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the demand).

OLIVE, Mark R
For and on behalf of
the applicant/s

For International Preliminary Examining Authority use only

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2. Adjusted date of receipt of demand due to CORRECTIONS under Rule 60.1(b):

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4. ☐ The date of receipt of the demand is WITHIN the period of 19 months from the priority dated as extended by virtue of Rule 80.5.

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WO 00/69903
PCT/AU00/00436

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

To:
OLIVE, Mark, R
Davies Collison Cave
1 Little Collins Street
Melbourne, Victoria 3000
AUSTRALIE

TUESDAY, - 5 DEC 2000

Date of mailing (day/month/year) 23 November 2000 (23.11.00)	
Applicant's or agent's file reference 2290375/MRO	**IMPORTANT NOTICE**

International application No.	**International filing date** (day/month/year)	**Priority date** (day/month/year)
PCT/AU00/00436	11 May 2000 (11.05.00)	13 May 1999 (13.05.99)

Applicant
PFIZER PRODUCTS INC et al

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this Notice:
   AG,AU,DZ,KP,KR,US

   In accordance with Rule 47.1(c), third sentence, those Offices will accept the present Notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:
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   The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).

3. Enclosed with this Notice is a copy of the international application as published by the International Bureau on
   23 November 2000 (23.11.00) under No. WO 00/69903

REMINDER REGARDING CHAPTER II (Article 31(2)(a) and Rule 54.2)

If the applicant wishes to postpone entry into the national phase until 30 months (or later in some Offices) from the priority date, **a demand for international preliminary examination** must be filed with the competent International Preliminary Examining Authority before the expiration of 19 months from the priority date.

It is the applicant's sole responsibility to monitor the 19-month time limit.

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

REMINDER REGARDING ENTRY INTO THE NATIONAL PHASE (Article 22 or 39(1))

If the applicant wishes to proceed with the international application in the **national phase**, he must, within 20 months or 30 months, or later in some Offices, perform the acts referred to therein before each designated or elected Office.

For further important information on the time limits and acts to be performed for entering the national phase, see the Annex to Form PCT/IB/301 (Notification of Receipt of Record Copy) and Volume II of the PCT Applicant's Guide.

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Published
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(54) Title: *LAWSONIA* DERIVED GENE AND RELATED SodC POLYPEPTIDES, PEPTIDES AND PROTEINS AND THEIR USES

(57) Abstract

The present invention relates generally to therapeutic compositions for the treatment and/or prophylaxis of intestinal disease conditions in animals and birds caused or exacerbated by *Lawsonia intracellularis* or similar or otherwise related microorganism. In particular, the present invention provides a novel gene derived from *Lawsonia intracellularis* which encodes an immunogenic SodC peptide, polypeptide or protein that is particularly useful as an antigen in vaccine preparation for conferring humoral immunity against *Lawsonia intracellularis* and related pathogens in animal hosts. The present invention is also directed to methods for the treatment and/or prophylaxis of such intestinal disease conditions and to diagnostic agents and procedures for detecting *Lawsonia intracellularis* or similar or otherwise related microorganisms.

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LAWSONIA DERIVED GENE AND RELATED SodC POLYPEPTIDES, PEPTIDES AND PROTEINS AND THEIR USES

FIELD OF THE INVENTION

The present invention relates generally to therapeutic compositions for the treatment and/or prophylaxis of intestinal disease conditions in animals and birds caused or exacerbated by *Lawsonia intracellularis* or similar or otherwise related microorganism. In particular, the present invention provides a novel gene derived from *Lawsonia intracellularis* which encodes an immunogenic peptide, polypeptide or protein. The polypeptide described herein, designated as sodC, or a peptide homologue, analogue or derivative thereof is particularly useful as an antigen in vaccine preparation for conferring humoral immunity against *Lawsonia intracellularis* and related pathogens in animal hosts. The present invention is also directed to methods for the treatment and/or prophylaxis of such intestinal disease conditions and to diagnostic agents and procedures for detecting *Lawsonia intracellularis* or similar or otherwise related microorganisms.

GENERAL

Bibliographic details of the publications numerically referred to in this specification are collected at the end of the description. All patents, patent applications, and publications cited herein are incorporated by reference in their entirety.

Reference hereinafter to *"Lawsonia intracellularis"* or its abbreviation *"L. intracellularis"* includes all microorganisms similar to or otherwise related to this microorganism, as described by Stills (1991) or Jones *et al.*(1997) or Lawson *et al.* (1993) or McOrist *et al.* (1995).

As used herein, the word "*sodC*", or the term "*sodC* gene", shall be taken to refer to the gene encoding the SodC polypeptide of the present invention.

As used herein the term "derived from" shall be taken to indicate that a specified product, in particular a macromolecule such as a peptide, polypeptide, protein, gene

or nucleic acid molecule, antibody molecule, Ig fraction, or other macromolecule, or a biological sample comprising said macromolecule, may be obtained from a particular source, organism, tissue, organ or cell, albeit not necessarily directly from that source, organism, tissue, organ or cell.

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated step or element or integer or group of steps or elements or integers but not the exclusion of any other step or element or integer or group of elements or integers.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations or any two or more of said steps, features, compositions and compounds.

The present invention is not to be limited in scope by the specific embodiments described herein, which are intended for the purposes of exemplification only. Functionally equivalent products, compositions and methods are clearly within the scope of the invention, as described herein.

BACKGROUND OF THE INVENTION

The meat-producing sector of the agricultural industry is dependant upon the health of its livestock and there is a need to maintain disease-free livestock for human consumption. The industry is subject to rapid economic downturn in response to disease conditions adversely affecting livestock and the quality of meat products derived therefrom, including those diseases which may potentially be transmitted to humans. It is important, therefore, to have well defined treatments and prophylactic and diagnostic procedures available to deal with infections or potential infections in

livestock animals and humans.

Meat products derived from porcine and avian species are significant commercial products in the agriculture industry. In particular, pigs form a major component of the meat industry. However, pigs are sensitive to a wide spectrum of intestinal diseases collectively referred to as porcine proliferative enteropathy (PPE). These diseases have previously been known as intestinal adenomatosis complex (Barker and van Drumel, 1985), porcine intestinal adenomatosis (PIA), necrotic enteritis (Rowland and Lawson, 1976), proliferative haemorrhagic enteropathy (Love and Love, 1977), regional ileitis (Jonsson and Martinsson, 1976), haemorrhagic bowel syndrome (O'Neil, 1970), porcine proliferative enteritis and *Campylobacter* spp – induced enteritis (Straw, 1990).

There are two main forms of PPE: a non-haemorrhagic form represented by intestinal adenomatosis which frequently causes growth retardation and mild diarrhoea; and a haemorrhagic form, which is often fatal, represented by proliferative haemorrhagic enteropathy (PHE), where the distal small intestine lumen becomes engorged with blood. PPE has been reported in a number of animal species including pigs (McOrist *et al*, 1993), hamsters (Stills, 1991), ferrets (Fox *et al*, 1989), guinea pigs (Elwell *et al*, 1981), rabbits (Schodeb and Fox, 1990) as well as avian species (Mason *et al*, 1998).

The causative organism of PPE is a *Campylobacter*-like organism referred to herein as *"Lawsonia intracellularis"* (McOrist *et al*, 1995). The organism has also been previously referred to as *Ileal symbiont intracellularis* (Stills, 1991). PPE-like diseases in pigs may also be caused by other pathogens such as various species of *Campylobacter* (Gebhart *et al*, 1983).

Lawsonia intracellularis is an intracellular, possibly obligate intracellular, bacterium. It can only be cultured *in vitro* with tissue culture cells (Jones *et al.*, 1997; Lawson *et al.*, 1993; McOrist *et al*, 1995; International Patent Application No. PCT/US96/09576). *L. intracellularis* is located in the cytoplasm of the villus cells and intestinal crypt cells of infected animals. Pigs suffering from PPE are characterised by irregularities in the

villus cells and intestinal crypt structure with epithelial cell dysplasia, wherein crypt abscesses form as the villi and intestinal crypts become branched and fill with inflammatory cells.

PPE is a significant cost component associated with the pig industry, especially in terms of stock losses, medication costs, reduced growth rates of pigs and increased feed costs. PPE also contributes to downstream indirect costs in, for example, additional labour costs and environmental costs in dealing with antibiotic residue contamination, and in control measures to prevent the organism from being passed on or carried to other animals or humans.

Current control strategies for PPE rely on the use of antibacterials. However, such a strategy is considered to only be short to medium term, especially since governmental regulatory pressures tend to discourage animal husbandry practices which involve the use of prophylactic antibiotics. There is a need, therefore, to develop effective, safe and low cost alternatives to the use of antibiotics and, in particular, to develop vaccine preparations capable of conferring protective immunity against *Lawsonia intracellularis* infection in livestock animals.

The most effective vaccine preparations are generally comprised of a highly antigenic component, such as a peptide, polypeptide, protein or other macromolecule which is derived from the pathogenic organism against which the vaccine is directed, wherein said antigenic component produces little or no contraindications when administered to a susceptible host animal, and produces little or no antigenic cross-reactivity with desirable organisms, such as non-pathogenic organisms that are a part of the normal flora of the intestinal tract or other tissues of said host animal. In summary, an effective vaccine preparation must be immunogenic, specific and safe.

Accordingly, there is a need to identify highly immunogenic antigens produced by the bacterium *Lawsonia intracellularis*.

International Patent Application No. PCT/AU96/00767 describes several *L.*

intracellularis partial genetic sequences, and partial polypeptides encoded thereby. However, there is a need to further identify polypeptide immunogens produced by the bacterium *L. intracellularis* and immunogenic peptides derived therefrom, including those immunogens which are genus- or species-specific, for use in improved vaccine compositions. The presently-described invention provides such immunogens.

SUMMARY OF THE INVENTION

One aspect of the present invention is directed to an isolated or recombinant immunogenic polypeptide which comprises, mimics or cross-reacts with a B-cell or T-cell epitope of the SodC polypeptide derived from *Lawsonia spp*. Preferably, the isolated or recombinant immunogenic polypeptide is selected from the group consisting of the following:

(i) a peptide, oligopeptide or polypeptide which comprises an amino acid sequence which has at least about 70% sequence identity overall to the amino acid sequence set forth in SEQ ID NO:1;

(ii) a peptide, oligopeptide or polypeptide which comprises an amino acid sequence having at least about 50% sequence identity to about amino acid residues 1 to about 42 of SEQ ID NO:1;

(iii) a peptide, oligopeptide or polypeptide which comprises at least about 5 contiguous amino acids of the amino acid sequence defined by residues 1 to about 42 of SEQ ID NO:1; or

(iv) a homologue, analogue or derivative of (i) or (ii) or (iii), which mimics a B-cell or T-cell epitope of *Lawsonia spp*.

In a preferred embodiment, the polypeptide comprises or consists essentially of the amino acid sequence of SEQ ID NO:1, or about amino acids 1 to 42 thereof.

A further aspect of the present invention provides a vaccine composition for the prophylaxis or treatment of infection in an animal, such as a pig or bird, by *L. intracellularis* or a similar or otherwise related microorganism, said vaccine composition comprising an immunologically effective amount of an immunogenic component which comprises an isolated or recombinant polypeptide having at least about 70% overall

sequence identity to the amino acid sequence set forth in SEQ ID NO:1, or at least about 50% overall sequence identity to amino acid residues 1 to about 42 of SEQ ID NO:1 or comprising at least 5 contiguous amino acids in the region of positions 1 to about 42 of SEQ ID NO:1 or an immunogenic homologue, analogue or derivative thereof which is immunologically cross-reactive with *L. intracellularis*; and one or more carriers, diluents and/or adjuvants suitable for veterinary or pharmaceutical use.

In a preferred embodiment, the polypeptide of the vaccine composition comprises or consists essentially of the amino acid sequence of SEQ ID NO: 1 or about amino acids 1 to 42 thereof.

A further aspect of the invention extends to an immunologically interactive molecule, such as an antibody or antibody fragment, which is capable of binding to the immunogenic polypeptide of the invention.

A further aspect of the invention provides a method of diagnosing infection of an animal by *Lawsonia intracellularis* or a related microorganism, said method comprising the steps of contacting a biological sample derived from said animal with an immunologically interactive molecule of the present invention for a time and under conditions sufficient for a complex, such as an antigen:antibody complex, to form, and then detecting said complex formation.

A further aspect of the present invention contemplates a method of determining whether or not an animal has suffered from a past infection, or is currently infected, by *Lawsonia intracellularis* or a related microorganism, said method comprising contacting a tissue or fluid sample, such as blood or serum derived from said animal, with the immunogenic polypeptide of the invention for a time and under conditions sufficient for a complex, such as an antigen:antibody complex, to form, and then detecting said complex formation.

A further aspect of the present invention provides an isolated nucleic acid molecule which comprises a sequence of nucleotides that encodes, or is complementary to a

nucleic acid molecule that encodes, a peptide, oligopeptide or polypeptide selected from the following:

(i) a peptide, oligopeptide or polypeptide which comprises an amino acid sequence which has at least about 70% overall sequence identity to the amino acid sequence set forth in SEQ ID NO:1;

(ii) a peptide, oligopeptide or polypeptide which comprises an amino acid sequence having at least about 50% overall sequence identity to amino acid residues 1 to 42 of SEQ ID NO:1; or

(iii) a peptide, oligopeptide or polypeptide which comprises at least about 5 contiguous amino acids of the amino acid sequence defined by residues 1 to about 42 of SEQ ID NO:1; or

(iv) a homologue, analogue or derivative of (i) or (ii) or (iii), which mimics a B-cell or T-cell epitope of *Lawsonia spp*.

In a preferred embodiment, the isolated nucleic acid molecule comprises the nucleotide sequence set forth in SEQ ID NO:2, or at least that portion of SEQ ID NO: 2 encoding amino acid residues 1 to 42 of SEQ ID NO: 1 or a degenerate variant thereof, has at least about 50% sequence identity to all or a part thereof.

A still further aspect of the invention provides a diagnostic method of detecting *Lawsonia intracellularis* or related microorganism in a biological sample derived from an animal subject, said method comprising the steps of hybridising one or more polynucleotide or oligonucleotide probes or primers derived from the nucleotide sequence set forth in SEQ ID NO:2 or a complementary nucleotide sequence thereof or a homologue, analogue or derivative thereof, to said sample, and then detecting said hybridisation using a detection means. The detection means according to this aspect of the invention is any nucleic acid-based hybridisation or amplification reaction.

A further aspect of the invention provides an isolated probe or primer derived from SEQ ID NO:2 or a complementary nucleotide sequence thereto.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic representation of a deduced amino acid sequence alignment of various bacterial [Cu, Zn]-superoxide dismutase (SOD) polypeptides. Amino acid sequences of SodC from *Lawsonia intracellularis* (L.int), *Escherichia coli* (E.coli), *Salmonella typhimurium* (S.typhi), *Photobacterium leiognathi* (P.leio), *Haemophilus parainfluenzae* (H.para), *Brucella abortus* (B.abo), *Actinobacillus pleuropneumoniae* (A.pleu) and *Legionella pneumophila* (L.pneu) are shown aligned with each other. Gaps have been introduced to optimise the alignment. Positions containing identical amino acid with respect to the SodC sequence of *L. intracellularis* are shaded. Residues identical in all sequences appear in boldface. Leader peptide sequences are shown at the top of the Figure in the first grouping. The separation point of the leader peptide from the mature [Cu, Zn]-SodC polypeptide for *L. intracellularis* is proposed between Ala26 and Cys27. Amino acids that are recognised as critical for the function of bovine SOD (not shown) are each marked with an asterisk. The *L. intracellularis* SodC sequence (SEQ ID NO: 1) was deduced by translation of the nucleotide sequence of the *SodC* gene obtained from clone p98LI50.

DETAILED DESCRIPTION OF THE INVENTION

In work leading up to the present invention, the inventors sought to identify immunogenic proteins of *Lawsonia intracellularis* for use in vaccines for the prophylaxis and treatment of PPE in animals, including pigs and birds.

Accordingly, one aspect of the present invention is directed to an isolated or recombinant immunogenic polypeptide which comprises, mimics or cross-reacts with a B-cell or T-cell epitope of the SodC polypeptide derived from *Lawsonia spp.*

Epitopes of *Lawsonia spp.* may be B cell epitopes or T-cell epitopes. It is well-known that antibody-binding sites (B-cell epitopes) involve linear as well as conformational epitopes (van Regenmortel, 1992). B-cell epitopes are predominantly conformational. In contrast, T-cells recognize predominantly linear epitope sequences in combination with MHC class II molecules.

A precise identification and careful selection of epitopes of *Lawsonia spp.* facilitates the development of diagnostic reagents and vaccine compositions for the effective treatment or prophylaxis of *Lawsonia* infections. Epitope identification and characterization (i.e. determination of the molecular weight, amino acid sequence, and structure of epitopes of *Lawsonia spp.*) may be performed using art-recognised techniques. For the detection of conformational epitopes, degrading and denaturing of the epitope molecule must be avoided in order to conserve the three-dimensional structure, because the antigen-antibody reaction will be diminished if the secondary structure of the epitope is altered significantly. In practice, the characterisation and isolation of linear non-conformational epitopes is easier, because any immunoreactive regions are contained within a single peptide fragment or single amino acid sequence which is capable of being purified under a range of conditions.

Both non-conformational and conformational epitopes may be identified by virtue of their ability to bind detectable amounts of antibodies (such as IgM or IgG) from sera of animals immunised against or infected with *Lawsonia spp.* and, in particular *L. intracellularis,* or an isolated polypeptide derived therefrom or, alternatively, by virtue of their ability to bind detectable amounts of antibodies in a purified Ig fraction derived from such sera. The antibodies may be derived from or contained within pools of polyclonal sera, or may be monoclonal antibodies. Antibody fragments or recombinant antibodies, such as those expressed on the surface of a bacteriophage or virus particle, such as in a phage display library, may also be employed.

The determination of T-cell epitopes is performed by analysing the ability of the epitope peptides to induce the proliferation of peripheral blood lymphocytes or T-cell clones. The identification of T-cell epitopes is accomplished using a variety of methods as known in the art, including the use of whole and fragmented native or recombinant antigenic protein, as well as the more commonly employed "overlapping peptide" method. In the latter method, overlapping peptides which span the entire sequence of a polypeptide derived from *Lawsonia spp.* are synthesized and tested for their capacity to stimulate T-cell cytotoxic or proliferative responses *in vitro.*

Structure determination of both conformational non-linear and non-conformational linear epitopes may be performed by nuclear magnetic resonance spectroscopy (NMR) and X-ray crystallographic analysis. The determination of epitopes using X-ray techniques requires the protein-antibody complex to be crystallized, whereas NMR allows analysis of the complex in a liquid state. NMR measures the amount of amino acids as well as the neighbourhood of protons of different amino acid residues, wherein the alternating effect of two protons along the carbon backbone is characteristic of a particular epitope.

A successful method to recognize non-conformational linear epitopes is the immunoblot and in particular, the Western blot. Peptides may be generated from a complete *Lawsonia spp.* polypeptide by digestion with site-specific proteases, such as trypsin or chymotrypsin, and the peptides generated thereby can be separated using standard electrophoretic or chromatographic procedures. For example, after electrophoresis according to molecular weight using SDS/PAGE (SDS/polyacrylamide gel electrophoresis) and/or according to isoelectric point using IEF (isoelectric focussing) or alternatively, by two-dimensional electrophoresis, the peptides can be transferred to immobilizing nylon or nitrocellulose membranes and incubated with sera raised against the intact polypeptides. Peptides that comprise immunogenic regions (i.e., B-cell or T-cell epitopes) are bound by the antibodies in the sera and the bound antibodies may be detected using secondary antibodies, such as anti-IgG antibodies, that have been labelled radioactively or enzymatically. The epitopes may then be characterised by purification based upon their size, charge or ability to bind specifically to antibodies against the intact polypeptide, using one or more techniques, such as size-exclusion chromatography, ion-exchange chromatography, affinity chromatography or ELISA among others. After purification of the epitope, only one band or spot should be detectable with gel electrophoresis. The N-terminal or total sequencing of the peptide offers the possibility to compare the peptide with known proteins in databases.

Several computer-driven algorithms have now been devised to search for T-cell epitopes in proteins (Margalit *et al*, 1987; Vajda and C. DeLisi, 1990; Altuvia *et al.*,

1994; Parker *et al.*1994; DeGroot *et al.*,1995; Gabriel *et al*, 1995; Meister *et al.*, 1995). These algorithms search the amino acid sequence of a given protein for characteristics believed to be common to immunogenic peptides, locating regions that are likely to induce a cellular immune response *in vitro*. Computer-driven algorithms can identify regions of a *Lawsonia spp.* polypeptide that contain epitopes and are less variable among different isolates. Alternatively, computer-driven algorithms can rapidly identify regions of each isolate's more variable proteins that should be included in a multivalent vaccine.

The AMPHI algorithm (Margalit *et al.*,1987), which is based on the periodicity of T cell epitopes, has been widely used for the prediction of T-cell antigenic sites from sequence information alone. Essentially, AMPHI describes a common structural pattern of MHC binding motifs, since MHC binding motifs (i.e., patterns of amino acids that appear to be common to most of the peptides that bind to a specific MHC molecule) appear to exhibit the same periodicity as an alpha helix. Identification of T-cell epitopes by locating MHC binding motifs in an amino acid sequence provides an effective means of identifying immunogenic epitopes in diagnostic assays.

The EpiMer algorithm (Meister *et al.*, 1995; Gabriel *et al.*, 1995; DeGroot *et al.*, 1995) locates clustered MHC binding motifs in amino acid sequences of proteins, based upon the correlation between MHC binding motif-dense regions and peptides that may have the capacity to bind to a variety of MHC molecules (promiscuous or multi-determinant binders) and to stimulate an immune response in these various MHC contexts as well (promiscuous or multi-determinant epitopes). The EpiMer algorithm uses a library of MHC binding motifs for multiple class I and class II HLA alleles to predict antigenic sites within a protein that have the potential to induce an immune response in subjects with a variety of genetic backgrounds. EpiMer locates matches to each MHC-binding motif within the primary sequence of a given protein antigen. The relative density of these motif matches is determined along the length of the antigen, resulting in the generation of a motif-density histogram. Finally, the algorithm identifies protein regions in this histogram with a motif match density above an algorithm-defined cutoff density value, and produces a list of subsequences representing these

clustered, or motif-rich regions. The regions selected by EpiMer may be more likely to act as multi-determinant binding peptides than randomly chosen peptides from the same antigen, due to their concentration of MHC-binding motif matches. The selection of regions that are MHC binding motif-dense increases the likelihood that the predicted peptide contains a "valid" motif, and furthermore, that the reiteration of identical motifs may contribute to peptide binding.

Additional MHC binding motif-based algorithms have been described by Parker *et al.*(1994) and Altuvia *et al.*(1994). In these algorithms, binding to a given MHC molecule is predicted by a linear function of the residues at each position, based on empirically defined parameters, and in the case of the Altuvia *et al.*(1994) algorithm, known crystallographic structures may also be taken into consideration.

Recombinant methods offer the opportunity to obtain well characterized epitopes of high purity for the production of diagnostic reagents and epitope-specific vaccine formulations (Mohapatra *et al.*, 1995). Based upon the amino acid sequence of a linear epitope and identification of the corresponding nucleotide sequence encoding same, polymerase chain reaction (PCR) may be performed to amplify the epitope-encoding region from cDNA. After cloning and expression in a suitable vector/host system, a large amount of epitopes of high purity can be extracted. Accordingly, the present invention clearly extends to both isolated non-recombinant polypeptides and recombinant polypeptides in an impure or isolated form.

The term "polypeptide" as used herein shall be taken to refer to any polymer consisting of amino acids linked by covalent bonds and includes within its scope full-length proteins and parts or fragments thereof such as, for example, oligopeptides and short peptide sequences consisting of at least about 5 amino acid residues, preferably at least about 10 amino acid residues, more preferably at least about 12 amino acid residues, and even more preferably at least about 15 amino acid residues. Also included within the scope of the definition of a "polypeptide" are amino acid sequence variants, containing one or more preferably conservative amino acid substitutions,

deletions, or insertions, which do not alter at least one essential property of said polypeptide such as, for example, its immunogenicity, use as a diagnostic reagent, or effectiveness as a peptide vaccine against *Lawsonia spp*, amongst others. Accordingly, a polypeptide may be isolated from a source in nature, or chemically synthesized. Furthermore, a polypeptide may be derived from a full-length protein by chemical or enzymatic cleavage, using reagents such as CNBr, trypsin, or chymotrypsin, amongst others.

Conservative amino acid substitutions are well-known in the art. For example, one or more amino acid residues of a native SodC polypeptide of the present invention can be substituted conservatively with an amino acid residue of similar charge, size or polarity, with the resulting polypeptide retaining an ability to function in a vaccine or as a diagnostic reagent as described herein. Rules for making such substitutions include those described by Dayhof (1978). More specifically, conservative amino acid substitutions are those that generally take place within a family of amino acids that are related in their side chains. Genetically-encoded amino acids are generally divided into four groups: (1) acidic=aspartate, glutamate; (2) basic=lysine, arginine, and histidine; (3) non-polar=alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, and tryptophan; and (4) uncharged polar= glycine, asparagine, glutamine, cysteine, serine, threonine, and tyrosine. Phenylalanine, tyrosine and tryptophan are also jointly classified as aromatic amino acids. One or more replacements within any particular group such as, for example, the substitution of leucine for isoleucine or valine or alternatively, the substitution of aspartate for glutamate or threonine for serine, or of any other amino acid residue with a structurally-related amino acid residue, will generally have an insignificant effect on the function of the resulting polypeptide.

The present invention is not limited by the source of the subject immunogen and clearly extends to isolated and recombinant polypeptides which are derived from a natural or a non-natural occurring source.

The term "recombinant polypeptide" as used herein shall be taken to refer to a polypeptide which is produced *in vitro* or in a host cell by the expression of a genetic

sequence encoding said polypeptide, which genetic sequence is under the control of a suitable promoter, wherein a genetic manipulation has been performed in order to achieve said expression. Accordingly, the term "recombinant polypeptide" clearly encompasses polypeptides produced by the expression of genetic sequences contained in viral vectors, cosmids or plasmids that have been introduced into prokaryotic or eukaryotic cells, tissues or organs. Genetic manipulations which may be used in this context will be known to those skilled in the art and include, but are not limited to, nucleic acid isolation, restriction endonuclease digestion, exonuclease digestion, end-filling using the Klenow fragment of *E. coli* DNA polymerase I or T4 DNA polymerase enzymes, blunt-ending of DNA molecules using T4 DNA polymerase or ExoIII enzymes, site-directed mutagenesis, ligation, and amplification reactions. As will be known to those skilled in the art, additional techniques such as nucleic acid hybridisations and nucleotide sequence analysis may also be utilised in the preparation of recombinant polypeptides, in confirming the identity of a nucleic acid molecule encoding a desired recombinant polypeptide and a genetic construct comprising the nucleic acid molecule.

Wherein the polypeptide of the present invention is a recombinant polypeptide, it may be produced in and, if desirable, isolated from a recombinant viral vector expression system or host cell. As will be known to those skilled in the relevant art, a cell for production of a recombinant polypeptide is selected on the basis of several parameters including the genetic constructs used to express the polypeptide under consideration, as well as the stability and activity of said polypeptide. It will also be known to those skilled in the art, that the stability or activity of a recombinant polypeptide may be determined at least in part, by post-translational modifications to the polypeptide such as, for example, glycosylation, acylation or alkylation reactions, amongst others, which may vary between cell lines used to produce the recombinant polypeptide.

Accordingly, in a more particularly preferred embodiment, the present invention extends to a recombinant polypeptide or a derivative, homologue or analogue thereof as present in a virus particle, or as produced in prokaryotic or eukaryotic host cell, or in a virus or cell culture thereof.

The present invention also extends to a recombinant polypeptide according to any of the foregoing embodiments which is produced in a bacterial cell belonging to the genus *Lawsonia*, in particular a cell of *L. intracellularis,* or a culture thereof.

The term "isolated polypeptide" refers to a polypeptide of the present invention which has been purified to some extent, preferably to at least about 20% by weight of protein, preferably to at least about 50% by weight of protein, more preferably to at least about 60% by weight of protein, still more preferably to at least about 70% by weight of protein and even more preferably to at least about 80% by weight of protein or greater, from its natural source or, in the case of non-naturally-occurring polypeptides, from the culture medium or cellular environment in which it was produced. Such isolation may be performed to improve the immunogenicity of the polypeptide of the present invention, or to improve the specificity of the immune response against that polypeptide, or to remove toxic or undesirable contaminants therefrom. The necessary or required degree of purity of an isolated polypeptide will vary depending upon the purpose for which the polypeptide is intended, and for many applications it will be sufficient for the polypeptide preparation to contain no contaminants which would reduce the immunogenicity of the polypeptide when administered to a host animal, in particular a porcine or avian animal being immunized against PPE or, alternatively, which would inhibit immuno-specific binding in an immunoassay for the diagnosis of PPE or a causative agent thereof.

The purity of an isolated polypeptide of the present invention may be determined by any means known to those skilled in the art, including the degree of homogeneity of a protein preparation as assessed by SDS/polyacrylamide gel electrophoresis, 2-dimensional electrophoresis, or amino acid composition analysis or sequence analysis.

Preferably, the polypeptide of the present invention will be substantially homogeneous or substantially free of nonspecific proteins, as assessed by SDS/polyacrylamide gel electrophoresis, 2-dimensional electrophoresis, or amino acid composition analysis or sequence analysis.

The polypeptide of the present invention can be purified for use as a component of a vaccine composition by any one or a combination of methods known to those of ordinary skill in the art, including, for example, reverse phase chromatography, HPLC, ion-exchange chromatography, and affinity chromatography, among others.

In a preferred embodiment, the isolated or recombinant polypeptide of the invention possesses the enzymatic or biological activity of a SOD polypeptide such as, for example, the *L. intracellularis* SodC polypeptide, or is at least derived from a SodC polypeptide or, alternatively, is immunologically cross-reactive with the *L. intracellularis* SodC polypeptide of the present invention.

In a particularly preferred embodiment, the isolated or recombinant polypeptide of the invention is derived from *Lawsonia spp.* or other pathogenic agent associated with the onset and/or development of PPE and more preferably, the subject polypeptide is derived from *Lawsonia intracellularis*.

A B-cell or T-cell epitope of a SodC polypeptide or a derivative, homologue or analogue thereof may comprise any combination of the following:

(i) the primary amino acid sequence of a SodC polypeptide, known in the art as a continuous non-conformational epitope;

(ii) the secondary structure which a SodC polypeptide adopts, known in the art as a continuous conformational epitope;

(iii) the tertiary structure which a SodC polypeptide adopts in contact with another region of the same polypeptide molecule, known in the art as a discontinuous conformational epitope; or

(iv) the quaternary structure which a SodC polypeptide adopts in contact with a region of another polypeptide molecule, known in the art as a discontinuous conformational epitope.

Accordingly, immunogenic polypeptides or derivatives, homologues or analogues thereof comprising the same, or substantially the same primary amino acid sequence

are hereinafter defined as "immunogens which comprise a B-cell or T-cell epitope" or similar term.

Immunogenic polypeptides or derivatives, homologues, or analogues thereof comprising different primary amino acid sequences may comprise immunologically identical immunogens, because they possess conformational B-cell or T-cell epitopes that are recognised by the immune system of a host species to be identical. Such immunogenic polypeptides or derivatives, homologues or analogues thereof are hereinafter defined as "immunogens which mimic or cross-react with a B-cell or T-cell epitope", or similar term.

Accordingly, the present invention extends to an immunogen which comprises, mimics, or cross-reacts with a B-cell or T-cell epitope of an isolated or recombinant polypeptide according to any one of the foregoing embodiments or a derivative, homologue or analogue thereof. In a particularly preferred embodiment, the present invention provides an immunogen which comprises, mimics, or cross-reacts with a B-cell or T-cell epitope of an isolated or recombinant polypeptide which in its native form is obtainable from a species of *Lawsonia* such as, but not limited to *L. intracellularis,* and which polypeptide preferably possesses SodC activity.

Preferably, such immunogenic polypeptides will not comprise a primary amino acid sequence which is highly-conserved between *L. intracellularis* and another non-pathogenic microorganism which is normally resident in the gut or other organ of an animal, in particular a porcine or avian animal. The significance of this exclusion to those embodiments of the invention wherein specificity is essential to performance (eg vaccine and diagnostic applications) will be apparent to those skilled in the art.

To improve the immunogenicity of a subject polypeptide of the present invention one or more amino acids not corresponding to the original protein sequence can be added to the amino or carboxyl terminus of the polypeptide. Such extra amino acids are useful for coupling the polypeptide to another peptide or polypeptide, to a large carrier protein or to a solid support. Amino acids that are useful for these purposes include

but are not limited to tyrosine, lysine, glutamic acid, aspartic acid, cysteine and derivatives thereof. Additional protein modification techniques can be used such as, e.g., NH_2-acetylation or COOH-terminal amidation, to provide additional means for coupling the polypeptide to another polypeptide, protein, or peptide molecule, or to a solid support. Procedures for coupling polypeptides to each other, or to carrier proteins or solid supports, are well known in the art. Polypeptides containing the above-mentioned extra amino acid residues at either the carboxyl- or amino-termini and either uncoupled or coupled to a carrier or solid support, are consequently within the scope of the present invention.

Furthermore, the polypeptide can be immobilised to a polymeric carrier or support material.

In an alternative embodiment, the immunogenicity of a polypeptide of the present invention may be improved using molecular biology techniques to produce a fusion protein containing one or more polypeptides of the present invention fused to a carrier molecules such as a highly immunogenic protein. For example, a fusion protein containing a polypeptide of the present invention fused to the highly immunogenic B subunit of cholera toxin can be used to increase the immune response to the polypeptide. The present invention also contemplates fusion proteins comprising a cytokine, such as an interleukin, fused to the subject polypeptide of the present invention, and genes encoding same.

Preferably, the polypeptide of the present invention, or a derivative, homologue or analogue thereof, when administered to a mammal, induces an immune response in said mammal. More preferably, the polypeptide of the present invention, when administered to a mammal, in particular a porcine animal (e.g., a pig) induces a protective immune response against *Lawsonia spp.*, and preferably against *L. intracellularis,* therein. As used herein, the phrase "induction of a protective immune response", and the like, refers to the ability of the administered polypeptide of the present invention to prevent or detectably slow the onset, development, or progression of symptoms associated with *Lawsonia* infection, and preferably, to prevent or

detectably slow the onset, development, or progression of symptoms associated with PPE in pigs.

Preferably, the immunogenic polypeptide of the invention comprises an amino acid sequence which is substantially the same as the amino acid sequence set forth in SEQ ID NO:1 or is at least about 75% identical overall to SEQ ID NO:1, or is at least about 75% identical to at least 8 contiguous amino acids of SEQ ID NO:1. In a preferred embodiment, the immunogenic polypeptide of the present invention consists essentially of the amino acid sequence of SEQ ID NO:1, or the amino acid sequence encoded by the SodC-encoding nucleotide sequence present in pALK14 (ATCC 207155) or about the first forty two amino acids thereof.

For the purposes of nomenclature, the amino acid sequence set forth in SEQ ID NO:1 represents the partial amino acid sequence of the SodC polypeptide comprising the N-terminal region, encoded by the 5′-end of the *Lawsonia intracellularis sodC* gene. The nucleotide sequence of the 5′-end of the *sodC* gene is set forth in SEQ ID NO:2.

Preferably, the percentage amino acid sequence identity to SEQ ID NO:1 is at least about 80%, more preferably at least about 85%, even more preferably at least about 90%, and still even more preferably at least about 95% identical to SEQ ID NO:1.

In determining whether or not two amino acid sequences fall within these percentage limits, those skilled in the art will be aware that it is necessary to conduct a side-by-side comparison or multiple alignment of sequences. In such comparisons or alignments, differences will arise in the positioning of non-identical residues, depending upon the algorithm used to perform the alignment. In the present context, reference to a percentage sequence identity or similarity between two or more amino acid sequences shall be taken to refer to the number of identical and similar residues respectively, between said sequences as determined using any standard algorithm known to those skilled in the art. For example, amino acid sequence identities or similarities may be calculated using the GAP programme of the Computer Genetics Group, Inc., University Research Park, Madison, Wisconsin, United States of America (Devereaux *et al*,

1984). The GAP programme utilizes the algorithm of Needleman and Wunsch (1970) to maximise the number of identical/similar residues and to minimise the number and/or length of sequence gaps in the alignment. Alternatively or in addition, where more than two amino acid sequences are being compared, the ClustalW programme of Thompson *et al* (1994) can be used.

In an alternative embodiment, the present invention provides an isolated or recombinant immunogenic polypeptide which comprises, mimics or cross-reacts with a B-cell or T-cell epitope of the SodC polypeptide derived from *Lawsonia spp.* wherein said isolated or recombinant immunogenic polypeptide comprises an amino acid sequence which comprises at least 5 contiguous amino acid residues of SEQ ID NO:1 or a homologue, analogue or derivative thereof.

Preferably, the isolated or recombinant immunogenic polypeptide of the invention comprises at least about 10 contiguous amino acids derived from SEQ ID NO:1, more preferably at least about 20 contiguous amino acid residues derived from SEQ ID NO:1, even more preferably at least about 30 contiguous amino acid residues derived from SEQ ID NO:1 and still even more preferably at least about 40 contiguous amino acid residues derived from SEQ ID NO:.

The present invention further encompasses homologues, analogues and derivatives of a polypeptide comprising the amino acid sequence set forth in SEQ ID NO:1.

"Homologues" of a polypeptide are those polypeptides which contain amino acid substitutions, deletions and/or additions relative to the polypeptide without altering one or more of its properties, such as its immunogenicity, biological activity or catalytic activity. In such molecules, amino acids can be replaced by other amino acids having similar properties such as, for example, hydrophobicity, hydrophilicity, hydrophobic moment, antigenicity, propensity to form or break α-helical structures of β-sheet structures, and so on.

Substitutional variants are those in which at least one residue in the sequence has

been removed and a different residue inserted in its place. Amino acid substitutions are typically of single residues, but may be clustered depending upon functional constraints placed upon the polypeptide; insertions will usually be of the order of about 1-10 amino acid residues. and deletions will range from about 1-20 residues. Preferably, amino acid substitutions will comprise conservative amino acid substitutions, such as those described *supra*.

Insertional amino acid sequence variants are those in which one or more amino acid residues are introduced into a predetermined site in the protein. Insertions can comprise amino-terminal and/or carboxyl terminal fusions as well as intra-sequence insertions of single or multiple amino acids. Generally, insertions within the amino acid sequence will be smaller than amino or carboxyl terminal fusions, of the order of about 1 to 4 residues.

Deletional variants are characterised by the removal of one or more amino acids from the sequence.

Amino acid variants of the polypeptide of the present invention may readily be made using peptide synthetic techniques well known in the art, such as solid phase peptide synthesis and the like, or by recombinant DNA manipulations. The manipulation of DNA sequences to produce variant proteins which manifest as substitutional, insertional or deletional variants are well known in the art. For example, techniques for making substitution mutations at predetermined sites in DNA having known sequence are well known to those skilled in the art, such as by M13 mutagenesis or other site-directed mutagenesis protocol.

"Analogues" are defined as peptides, oligopeptides and polypeptides which are functionally equivalent to the peptides of the present invention but which contain certain non-naturally occurring or modified amino acid residues as will be known to those skilled in the art. Accordingly, an "analogue" as defined herein need not comprise an amino acid sequence which is similar to the amino acid sequence set forth herein such as, for example, peptides, oligopeptides and polypeptides which are

derived from computational predictions or empirical data revealing the secondary, tertiary or quaternary structure of the polypeptide of the present invention, and which therefore do not comprise the same primary amino acid sequence of said polypeptide, yet nevertheless mimic or cross-react with B-cell or T-cell epitope of *Lawsonia spp.* and preferably, mimic or cross-react with B-cell or T-cell epitope of *Lawsonia intracellularis.*

For example, mimotopes (polypeptide analogues that cross-react with a B-cell or T-cell epitope of the *Lawsonia* polypeptide of the invention but, however, comprise a different amino acid sequence to said epitope) may be identified by screening random amino acid sequences in peptide libraries with antibodies that bind to a desired T-cell or B-cell epitope. As with techniques for the identification of B-cell or T-cell epitopes as described *supra*, the antibodies used to identify such mimotopes may be polyclonal or monoclonal or recombinant antibodies, in crude or purified form. Mimotopes of a T-cell epitope may then be assayed further for their ability to stimulate T-cell cytotoxic or proliferative responses *in vitro*. Mimotopes are particularly useful as analogues of non-linear (i.e., conformational) epitopes of the polypeptide of the present invention, because conformational epitopes are generally formed from non-contiguous regions in a polypeptide, and the mimotopes provide immunogenic equivalents thereof in the form of a single peptide molecule.

Additionally, the use of polypeptide analogues can result in polypeptides with increased immunogenic and/or antigenic activity, that are less sensitive to enzymatic degradation, and which are more selective. A suitable proline analogue is 2-aminocyclopentane carboxylic acid (βAC^5c) which has been shown to increase the immunogenic activity of a native polypeptide more than 20 times (Mierke *et al*, 1990; Portoghese *et al*, 1990; Goodman *et al*, 1987).

"Derivatives" of a polypeptide described herein are those peptides, oligopeptides and polypeptides which comprise at least about five contiguous amino acid residues of the amino acid sequence set forth in SEQ ID NO:1. A "derivative" may further comprise additional naturally-occurring, altered glycosylated, acylated or non-naturally occurring

amino acid residues compared to the amino acid sequence set forth in SEQ ID NO:1. Alternatively or in addition, a derivative may comprise one or more non-amino acid substituents such as, for example, a reporter molecule or other ligand, covalently or non-covalently bound to the amino acid sequence such as, for example, a reporter molecule which is bound thereto to facilitate its detection.

Other examples of recombinant or synthetic mutants and derivatives of the peptide immunogens of the present invention include those incorporating single or multiple substitutions, deletions and/or additions therein, such as carbohydrates, lipids and/or proteins or polypeptides. Naturally occurring or altered glycosylated or acylated forms of the subject peptides are particularly contemplated by the present invention. Additionally, homopolymers or heteropolymers comprising one or more copies of the subject peptide listed in SEQ ID NO:1, or one or more derivatives, homologues or analogues thereof, are within the scope of the invention.

Preferably, homologues, analogues and derivatives of the polypeptide of the invention are "immunogenic", defined hereinafter as the ability of said polypeptide, or a derivative, homologue or analogue thereof, to elicit B cell and/or T cell responses in the host, in response to immunization.

Preferred homologues, analogues and derivatives of the amino acid sequence set forth in SEQ ID NO:1 include those amino acid variants that function as B cell or T cell epitopes of said amino acid sequence which are capable of mediating an immune response such as, for example, mimotopes of the immunogenic polypeptide described herein which have been produced by synthetic means, such as by Fmoc chemistry. The only requirement of such molecules is that they cross-react immunologically with a polypeptide which comprises the amino acid sequence set forth in SEQ ID NO:1 or the first 42 amino acid residues thereof or a derivative thereof which comprises at least 5 contiguous amino acids in length of SEQ ID NO:1.

As will be apparent to those skilled in the art, such homologues, analogues and derivatives of the polypeptide of the invention molecules will be useful to prepare

antibodies that cross-react with antibodies against said polypeptide and/or to elicit a protective immune response of similar specificity to that elicited by said polypeptide. Such molecules will also be useful in diagnostic and other applications that are immunological in nature such as, for example, diagnostics which utilise one or more immunoassay formats (eg. ELISA, RIA and the like).

Accordingly, the immunogen of the present invention or a derivative, homologue or analogue thereof is useful in vaccine compositions that protect an individual against infection by *L. intracellularis* and/or as an antigen to elicit polyclonal or monoclonal antibody production and/or in the detection of antibodies against *L. intracellularis* in infected animals, particularly in porcine and avian animals.

The present inventors have also shown that the N-terminal region of SEQ ID NO:1 is particularly unique, as compared to other immunogenic amino acid sequences, including those of the SodC polypeptides of other animal pathogens. Accordingly, peptides, oligopeptides and polypeptides which comprise such unique epitope regions of SEQ ID NO:1, will have improved specificity compared to other regions of the *Lawsonia spp.* SodC molecule. The particular advantages of such peptides will be immediately apparent to those skilled in the production of vaccine compositions, where specificity against a pathogen of interest is an important consideration.

In particular, the present inventors have shown that amino acids from about 1 to about 42 of the *Lawsonia intracellularis* SodC polypeptide, as set forth in SEQ ID NO:1, is not highly conserved compared to the corresponding region of the *Escherichia coli* SodC polypeptide, being only about 15% identical thereto. Accordingly, this region of the *L. intracellularis* SodC polypeptide is a promising antigenic peptide for the formulation of *Lawsonia*-specific vaccines and diagnostics for the specific detection of *Lawsonia spp.* in biological samples.

Accordingly, in an alternative embodiment, the present invention provides an isolated or recombinant immunogenic polypeptide or a derivative, homologue or analogue thereof which comprises, mimics or cross-reacts with a B-cell or T-cell epitope of a

Lawsonia spp. wherein said polypeptide comprises a sequence of amino acids which has at least about 50% sequence identity to about amino acid residues 1 to about amino acid residue 42 of the *L. intracellularis* SodC polypeptide as set forth in SEQ ID NO:1. Preferably, the percentage sequence identity to amino acids 1 to about 42 of SEQ ID NO:1 is at least about 60%, more preferably at least about 70%, even more preferably at least about 80% and still even more preferably at least about 90%. In a particularly preferred embodiment, the subject polypeptide will comprise a sequence of amino acids from about amino acid 1 to about amino acid 42 of SEQ ID NO:1.

A second aspect of the present invention provides a vaccine composition for the prophylaxis or treatment of infection in a mammal or bird by *L. intracellularis* or similar or otherwise related microorganism, said vaccine composition comprising:

(i) an immunogenic component which comprises an isolated or recombinant polypeptide having at least about 70% overall amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1 and/or at least about 50% amino acid sequence identity to amino acid residues 1 to about 42 of SEQ ID NO:1 or comprising at least 5 contiguous amino acids derived from SEQ ID NO:1 or an immunogenic homologue, analogue or derivative thereof which is immunologically cross-reactive with *Lawsonia intracellularis;* and

(ii) one or more carriers, diluents and/or adjuvants suitable for veterinary or pharmaceutical use.

As used herein, the term "immunogenic component" refers to a peptide, polypeptide or a protein encoded by DNA from, or derived from, *L. intracellularis* or a related microorganism thereto which is capable of inducing a protective immune response in an animal, in particular a porcine or avian animal, whether or not said peptide, polypeptide or protein is in an isolated or recombinant form. Accordingly, the vaccine composition clearly encompasses those vaccine compositions which comprise attenuated, killed or non-pathogenic isolates or forms of *L. intracellularis* or related microorganisms thereto which comprise or express said peptide, polypeptide or protein.

By "protective immune response" is meant that the immunogenic component elicits an immune response in the animal to which the vaccine composition is administered at the humoral and/or cellular level which is sufficient to prevent infection by *Lawsonia intracellularis* or a related microorganism thereto and/or which is sufficient to detectably reduce one or more symptoms or conditions, or to detectably slow the onset of one or more symptoms or conditions, associated with infection by *Lawsonia intracellularis* or a related microorganism thereto in an animal host, as compared to a control infected animal. The term "effective amount" of an immunogenic component present in the vaccine composition refers to that amount of said immunogenic component that is capable of inducing a protective immune response after a single complete dose has been administered, or after several divided doses have been administered.

Preferably, the polypeptide component of the subject vaccine composition comprises an amino acid sequence which is both immunogenic and specific, by virtue of its immunological cross-reactivity with the causative agent of PPE, *Lawsonia intracellularis*. In this regard, it will be apparent from the preceding description that such polypeptide components may comprise an amino acid sequence derived from SEQ ID NO:1 or a homologue, analogue or derivative of the amino acid sequence set forth in SEQ ID NO:1 such as, for example, a mimotope of said sequence.

The immunogenic polypeptide or immunogenic homologue, analogue or derivative may be a naturally-occurring peptide, oligopeptide or polypeptide in isolated or recombinant form according to any of the embodiments described *supra* or exemplified herein. Preferably, the immunogenic polypeptide or immunogenic homologue, analogue or derivative is derived from *Lawsonia spp.*, in particular *L. intracellularis* or a microorganism that is related thereto.

Preferably, the immunogenic component has undergone at least one purification step or at least partial concentration from a cell culture comprising *L. intracellularis* or a related microorganism thereto, or from a lysed preparation of *L. intracellularis* cells or related microorganism, or from another culture in which the immunogenic component is recombinantly expressed. The purity of such a component which has the requisite

immunogenic properties is preferably at least about 20% by weight of protein in a particular preparation, more preferably at least about 50%, even more preferably at least about 60%, still more preferably at least about 70% and even more preferably at least about 80% or greater.

The immunogenic component of the vaccine of the present invention can comprise a single peptide, polypeptide or protein, or a range or combination of different peptides, polypeptides or proteins covering different or similar epitopes. In addition or, alternatively, a single polypeptide can be provided with multiple epitopes. The latter type of vaccine is referred to as a polyvalent vaccine. A multiple epitope includes two or more epitopes located within a peptide or polypeptide molecule.

The formulation of vaccines is generally known in the art and reference can conveniently be made to Remington's Pharmaceutical Sciences, 17th ed., Mack Publishing Co., Easton, Pennsylvania, USA.

A particularly useful form of the vaccine is a recombinant vaccine produced, for example, in a vaccine vector, such as but not limited to a cell transfected with a vaccinia virus vector or a bacterial cell capable of expressing the immunogenic component.

The present invention clearly extends to recombinant vaccine compositions in which the immunogenic component at least is contained within killed vaccine vectors prepared, for example, by heat, formalin or other chemical treatment, electric shock or high or low pressure forces. According to this embodiment, the immunogenic component of the vaccine is generally synthesized in a live vaccine vector which is killed prior to administration to an animal.

Furthermore, the vaccine vector expressing the immunogenic component may be non-pathogenic or attenuated. Within the scope of this embodiment are cells that have been transfected with non-pathogenic or attenuated viruses encoding the immunogenic component of the vaccine and non-pathogenic or attenuated cells that

directly express the immunogenic component.

Attenuated or non-pathogenic host cells include those cells which are not harmful to an animal to which the subject vaccine is administered. As will be known to those skilled in the art, "live vaccines" can comprise an attenuated virus vector encoding the immunogenic component or a host cell comprising same, which is capable of replicating in an animal to which it is administered, and using host cell machinery to express the immunogenic component albeit producing no adverse side-effects therein. Such vaccine vectors may colonise the gut or other organ of the vaccinated animal. Such live vaccine vectors are efficacious by virtue of their ability to continually express the immunogenic component in the host animal for a time and at a level sufficient to confer protective immunity against a pathogen which expresses an immunogenic equivalent of said immunogenic component. The present invention clearly encompasses the use of such attenuated or non-pathogenic vectors and live vaccine preparations.

The vaccine vector may be a virus, bacterial cell or a eukaryotic cell such as an avian, porcine or other mammalian cell or a yeast cell or a cell line such as COS, VERO, HeLa, mouse C127, Chinese hamster ovary (CHO), WI-38, baby hamster kidney (BHK) or MDCK cell lines. Suitable prokaryotic cells include *Mycobacterium spp., Corynebacterium spp., Salmonella spp., Escherichia coli, Bacillus spp.* and *Pseudomonas spp*, amongst others. Bacterial strains which are suitable for the present purpose are well-known in the relevant art (Ausubel *et al*, 1987; Sambrook *et* al, 1989).

Such cells and cell lines are capable of expression of a genetic sequence encoding a SodC peptide, polypeptide or protein of the present invention from *L. intracellularis* in a manner effective to induce a protective immune response in the animal. For example, a non-pathogenic bacterium could be prepared containing a recombinant sequence capable of encoding a peptide, polypeptide or protein from *L. intracellularis*. The recombinant sequence would be in the form of an expression vector under the control of a constitutive or inducible promoter. The bacterium would then be permitted

to colonise suitable locations in a pig's gut and would be permitted to grow and produce the recombinant peptide, polypeptide or protein in amount sufficient to induce a protective immune response against *L. intracellularis*.

In a further alternative embodiment, the vaccine can be a DNA or RNA vaccine comprising a DNA or RNA molecule encoding a peptide, polypeptide or protein of the present invention which is injected into muscular tissue or other suitable tissue in a pig under conditions sufficient to permit transient expression of said DNA or RNA to produce an amount of peptide, polypeptide or protein effective to induce a protective immune response. In a preferred embodiment, the DNA vaccine is in the form of a plasmid in which the DNA is operably connected with a promoter region capable of expressing the nucleotide sequence encoding the immunogen in cells of the immunized animal.

In the production of a recombinant vaccine, except for a DNA vaccine described herein, it is therefore necessary to express the immunogenic component in a suitable vector system. For the present purpose, the immunogenic component can be expressed by:

(i) placing an isolated nucleic acid molecule in an expressible format, said nucleic acid molecule comprising the coding region of the nucleotide sequence set forth in SEQ ID NO:2 or a protein-encoding homologue, analogue or derivative of SEQ ID NO:2 selected from the group consisting of:

(a) nucleotide sequences that have at least about 70% sequence identity to SEQ ID NO:2;

(b) nucleotide sequences that hybridise under at least low stringency hybridisation, preferably under at least moderate stringency conditions, and even more preferably under high stringency conditions, to the complement of SEQ ID NO:2; and

(c) nucleotide sequences that encode the amino acid sequence set forth in SEQ ID NO:1 or a homologue, analogue or derivative thereof, including, for example, a mimotope of the amino acid set forth in SEQ ID NO:1;

(ii) introducing the isolated nucleic acid molecule of (i) in an expressible format into a suitable vaccine vector; and

(iii) incubating or growing the vaccine vector for a time and under conditions sufficient for expression of the immunogenic component encoded by said nucleic acid molecule to occur.

For the purposes of defining the level of stringency, a low stringency is defined herein as being a hybridisation and/or a wash carried out in 6xSSC buffer, 0.1% (w/v) SDS at 28°C. A moderate stringency is defined herein as being a hybridisation and/or washing carried out in 2xSSC buffer, 0.1% (w/v) SDS at a temperature in the range 45°C to 65°C. A high stringency is defined herein as being a hybridisation and/or wash carried out in 0.1xSSC buffer, 0.1% (w/v) SDS at a temperature of at least 65°C.

Generally, the stringency is increased by reducing the concentration of SSC buffer, and/or increasing the concentration of SDS and/or increasing the temperature of the hybridisation and/or wash. Those skilled in the art will be aware that the conditions for hybridisation and/or wash may vary depending upon the nature of the hybridisation membrane or the type of hybridisation probe used. Conditions for hybridisations and washes are well understood by one normally skilled in the art. For the purposes of clarification of the parameters affecting hybridisation between nucleic acid molecules, reference is found in pages 2.10.8 to 2.10.16. of Ausubel *et al.* (1987), which is herein incorporated by reference.

As used herein, a "nucleic acid molecule in an expressible format" is a protein-encoding region of a nucleic acid molecule placed in operable connection with a promoter or other regulatory sequence capable of regulating expression in the vaccine vector system.

Reference herein to a "promoter" is to be taken in its broadest context and includes the transcriptional regulatory sequences of a classical genomic gene, including the TATA box which is required for accurate transcription initiation, with or without a CCAAT box sequence and additional regulatory elements (i.e., upstream activating sequences,

enhancers and silencers) which alter gene expression in response to developmental and/or external stimuli, or in a tissue-specific manner. In the present context, the term "promoter" is also used to describe a recombinant, synthetic or fusion molecule, or derivative which confers, activates or enhances the expression of a nucleic acid molecule to which it is operably connected, and which encodes the immunogenic polypeptide. Preferred promoters can contain additional copies of one or more specific regulatory elements to further enhance expression and/or to alter the spatial expression and/or temporal expression of the said nucleic acid molecule.

Placing a nucleic acid molecule under the regulatory control of i.e., "in operable connection with" a promoter sequence means positioning the said molecule such that expression is controlled by the promoter sequence. Promoters are generally, but not necessarily, positioned 5′ (upstream) to the genes that they control. In the construction of heterologous promoter/structural gene combinations it is generally preferred to position the promoter at a distance from the gene transcription start site that is approximately the same as the distance between that promoter and the gene it controls in its natural setting, i.e., the gene from which the promoter is derived. Furthermore, the regulatory elements comprising a promoter are usually positioned within 2 kb of the start site of transcription of the gene. As is known in the art, some variation in this distance can be accommodated without loss of promoter function. Similarly, the preferred positioning of a regulatory sequence element with respect to a heterologous gene to be placed under its control is defined by the positioning of the element in its natural setting, i.e., the genes from which it is derived. Again, as is known in the art, some variation in this distance can also occur.

The prerequisite for producing intact polypeptides in bacteria such as *E. coli* is the use of a strong promoter with an effective ribosome binding site. Typical promoters suitable for expression in bacterial cells such as *E. coli* include, but are not limited to, the *lacz* promoter, temperature-sensitive λ_L or λ_R promoters, T7 promoter or the IPTG-inducible *tac* promoter. A number of other vector systems for expressing the nucleic acid molecule of the invention in *E. coli* are well-known in the art and are described, for example, in Ausubel *et al* (1987) or Sambrook *et al* (1989). Numerous plasmids

with suitable promoter sequences for expression in bacteria and efficient ribosome binding sites have been described, such as for example, pKC30 (λ_L: Shimatake and Rosenberg, 1981), pKK173-3 (*tac*: Amann and Brosius, 1985), pET-3 (T7: Studier and Moffat, 1986), the pFLEX series of expression vectors (Pfizer Inc., CT, USA) or the pQE series of expression vectors (Qiagen, CA), amongst others. Typical promoters suitable for expression in viruses of eukaryotic cells and eukaryotic cells include the SV40 late promoter, SV40 early promoter and cytomegalovirus (CMV) promoter, CMV IE (cytomegalovirus immediate early) promoter amongst others.

Means for introducing the isolated nucleic acid molecule or a genetic construct comprising same into a cell for expression of the immunogenic component of the vaccine composition are well-known to those skilled in the art. The technique used for a given organism depends on the known successful techniques. Means for introducing recombinant DNA into animal cells include microinjection, transfection mediated by DEAE-dextran, transfection mediated by liposomes such as by using lipofectamine (Gibco, MD, USA) and/or cellfectin (Gibco, MD, USA), PEG-mediated DNA uptake, electroporation and microparticle bombardment such as by using DNA-coated tungsten or gold particles (Agracetus Inc., WI, USA) amongst others.

The immunogenic component of a vaccine composition as contemplated herein exhibits excellent therapeutic activity, for example, in the treatment and/or prophylaxis of PPE when administered in an amount which depends on the particular case. For example, for recombinant peptide molecules, from about 0.5 µg to about 20 mg may be administered, preferably from about 1 μg to about 10 mg, more preferably from about 10 μg to about 5 mg, and most preferably from about 50 μg to about 1 mg equivalent of the immunogenic component in a volume of about 1ml to about 5ml. For DNA vaccines, a preferred amount is from about 0.1 μg/ml to about 5 mg/ml in a volume of about 1 to about 5 ml. The DNA can be present in "naked" form or it can be administered together with an agent facilitating cellular uptake (e.g., in liposomes or cationic lipids). The important feature is to administer sufficient immunogen to induce a protective immune response. The above amounts can be administered as stated or calculated per kilogram of body weight. Dosage regime can be adjusted to

provide the optimum therapeutic response. For example, several divided doses can be administered or the dose can be proportionally reduced as indicated by the exigencies of the therapeutic situation. Booster administration may also be required.

The vaccine of the present invention can further comprise one or more additional immunomodulatory components such as, for example, an adjuvant or cytokine molecule, amongst others, that is capable of increasing the immune response against the immunogenic component. Non-limiting examples of adjuvants that can be used in the vaccine of the present invention include the RIBI adjuvant system (Ribi Inc., Hamilton, MT, USA), alum, mineral gels such as aluminium hydroxide gel, oil-in-water emulsions, water-in-oil emulsions such as, for example, Block co-polymer (CytRx, Atlanta GA, USA),QS-21 (Cambridge Biotech Inc., Cambridge MA, USA), SAF-M (Chiron, Emeryville CA, USA), AMPHIGEN® adjuvant, Freund's complete adjuvant; Freund's incomplete adjuvant; and Saponin, QuilA or other saponin fraction, monophosphoryl lipid A, and Avridine lipid-amine adjuvant. Other immunomodulatory agents that can be included in the vaccine include, for example, one or more cytokines, such as interferon and/or interleukin, or other known cytokines. Non-ionic surfactants such as, for example, polyoxyethylene oleyl ether and n-hexadecyl polyethylene ether may also be included in the vaccines of the present invention.

The vaccine composition can be administered in a convenient manner such as by oral, intravenous (where water soluble), intramuscular, subcutaneous, intranasal, intradermal or suppository routes or by implantation (e.g., using slow release techno'ogy). Depending on the route of administration, the immunogenic component may be required to be coated in a material to protect it from the action of enzymes, acids and other natural conditions which may inactivate it, such as those in the digestive tract.

The vaccine composition may also be administered parenterally or intraperitoneally. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof, or in oils. Under ordinary conditions of storage and use, these preparations can contain a preservative to prevent the growth of microorganisms. Alternatively, the

vaccine composition can be stored in lyophilised form to be rehydrated with an appropriate vehicle or carrier prior to use.

Pharmaceutical forms suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. In all cases the form must be fluid to the extent that easy syringability exists, unless the pharmaceutical form is a solid or semi-solid such as when slow release technology is employed. In any event, it must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms.

The carrier may be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol and liquid polyethylene glycol, and the like), suitable mixtures thereof and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents such as, for example,, parabens, chlorobutanol, phenol, sorbic acid, thimerosal and the like. In many cases, it will be preferable to include isotonic agents such as, for example,, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption such as, for example,, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active compound in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filter-sterilization. Generally, dispersions are prepared by incorporating the sterilized active ingredient into a sterile vehicle which contains the basic dispersion medium and the required other ingredients selected from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze-drying technique which yield a powder of the active ingredient plus any

additional desired ingredient from previously sterile-filtered solution thereof.

The present invention extends to vaccine compositions which confer protection against infection by one or more isolates or sub-types of *L. intracellularis* including those that belong to the same serovar or serogroup as *Lawsonia intracellularis*. The vaccine composition preferably also confers protection against infection by other species of the genus *Lawsonia* or other microorganisms related thereto; as determined at the nucleotide, biochemical, structural, physiological and/or immunointeractive level; the only requirement being that said other species or other microorganism expresses a polypeptide which is immunologically cross-reactive to the polypeptide of the invention described herein. For example, such related microorganisms may comprise genomic DNA which is at least about 70% identical overall to the genomic DNA of *Lawsonia intracellularis* as determined using standard genomic DNA hybridisation and analysis techniques.

The terms "serogroup" and "serovar" relate to a classification of microorganisms which is based upon serological typing data, in particular data obtained using agglutination assays such as the microscopic agglutination test (MAT). Those skilled in the art will be aware that serovar and serogroup antigens are a mosaic on the cell surface and, as a consequence there will be no strict delineation between bacteria belonging to a serovar and/or serogroup. Moreover, organisms which belong to different species may be classified into the same serovar or serogroup because they are indistinguishable by antigenic determination. As used herein, the term "serovar" means one or more *Lawsonia* strains which are antigenically-identical with respect to antigenic determinants produced by one or more loci. Quantitatively, serovars may be differentiated from one another by cross-agglutination absorption techniques. As used herein, the term "serogroup" refers to a group of *Lawsonia spp.* whose members cross-agglutinate with shared group antigens and do not cross-agglutinate with the members of other groups and, as a consequence, the members of a serogroup have more or less close antigenic relations with one another by simple cross-agglutination.

The present invention thus clearly extends to vaccine compositions for the treatment and/or prophylaxis of animals, in particular, vaccine compositions for the treatment and/or prophylaxis of porcine and/or avian species, against any bacterium belonging to the same serovar or serogroup as *Lawsonia intracellularis*. Preferably, such organisms will express a polypeptide having an amino acid sequence identity of at least about 70% overall with respect to SEQ ID NO:1 and/or at least about 50% with respect to amino acids 1 to about 42 of SEQ ID NO:1.

The present invention extends further to vaccine compositions capable of conferring protection against a "genetic variant" of *Lawsonia intracellularis*, the only requirement being that said variant expresses a polypeptide having an overall amino acid sequence identity of at least about 70% with respect to SEQ ID NO:1 and/or at least about 50% with respect to amino acids 1 to about 42 of SEQ ID NO:1 or a homologue, analogue or derivative thereof which is immunologically cross-reactive thereto. Genetic variants of *L. intracellularis* can be developed by mutation, recombination, conjugation or transformation of *L. intracellularis* or may occur naturally. It will be known to a person skilled in the art how to produce such derivatives.

In a particularly preferred embodiment, the vaccine composition of the invention is intended for or suitable for the prophylaxis and/or treatment of infection in a porcine or avian animal and more preferably, for prophylaxis and/or treatment of a porcine animal for infection by *L. intracellularis*.

In a particularly preferred embodiment, the vaccine composition of the invention is intended for or suitable for the prophylaxis and/or treatment of infection in a porcine or avian animal and more preferably, for prophylaxis and/or treatment of a porcine animal for infection by *L. intracellularis*.

Accordingly, the present invention clearly extends to the use of the immunogenic polypeptide of the invention according to any one of the preceding embodiments or as exemplified herein in the preparation of a medicament for the treatment and/or prophylaxis of PPE in animals, particularly porcine or avian animals.

The invention further extends to a method of treatment and/or prophylaxis of PPE in an animal such as an avian or porcine animal, said method comprising administering the vaccine composition or the immunogenic polypeptide of the invention as described or exemplified herein to said animal for a time and under conditions sufficient for an immune response to occur thereto. Preferably, in the case of administration of a vaccine composition, the immune response to the immunogen is a protective immune response.

Those skilled in the art will recognise the general applicability of the invention in vaccinating animals other than porcine and avian animals against *L. intracellularis* and/or related microorganisms. In the general application of the vaccine of the present invention, the only prerequisite is that the animal on which protection is conferred is capable of being infected with *Lawsonia intracellularis* and/or a related microorganism thereto and that, in the case of a related microorganism to *L. intracellularis,* said related microorganism expresses a B-cell or T-cell epitope which mimics or cross-reacts with the polypeptide component of the vaccine composition described herein. Animals which may be protected by the vaccine of the present invention include, but are not limited to, humans, primates, companion animals (e.g., cats, dogs), livestock animals (e.g., pigs, sheep, cattle, horses, donkeys, goats), laboratory test animals (e.g., mice, rats, guinea pigs, rabbits) and captive wild animals (e.g., kangaroos, foxes, deer). The present invention also extends to the vaccination of birds such as poultry birds, game birds and caged birds.

The present invention further extends to combination vaccines comprising an effective amount of a first immunogenic component comprising the polypeptide of the present invention combined with an effective amount of a second immunogenic component comprising one or more other antigens capable of protecting a porcine animal, or bird, against either *Lawsonia spp.* or another pathogen that infects and causes disease in said animal. In a preferred embodiment, the second immunogenic component is selected from the group consisting of the *L. intracellularis* autolysin, hemolysin, FlgE, and OmpH polypeptides and homologues, analogues or derivatives thereof, in particular immunogenic variants or derivatives thereof, and nucleic acid molecules

encoding same.

The isolated or recombinant SodC polypeptide of the invention or an immunologically-equivalent homologue, analogue or derivative thereof is also useful for the preparation of immunologically interactive molecules which are useful in the diagnosis of infection of an animal by *Lawsonia spp.*, in particular by *L. intracellularis* or a related organism thereto.

As used herein, the term "immunologically interactive molecule" includes antibodies and antibody derivatives and functional equivalents, such as a Fab, or a SCAB (single-chain antibody), any of which optionally can be conjugated to an enzyme, radioactive or fluorescent tag, amongst others. The only requirement of such immunologically interactive molecules is that they are capable of binding specifically to the immunogenic polypeptide of the present invention as hereinbefore described.

Accordingly, a further aspect of the invention extends to an immunologically interactive molecule which is capable of binding to any one or more of the following:

(i) a peptide, oligopeptide or polypeptide which comprises an amino acid sequence which has at least about 70% overall sequence identity to the amino acid sequence set forth in SEQ ID NO:1;

(ii) a peptide, oligopeptide or polypeptide which comprises an amino acid sequence which comprises an amino having at least about 50% overall sequence identity to amino acid residues 1 to 42 of SEQ ID NO:1;

(iii) a peptide comprising at least 5 contiguous amino acid residues derived from amino acids 1 to about 42 of SEQ ID NO:1; or

(iv) a homologue, analogue or derivative of (i) or (ii) or (iii) which mimics a B-cell or T-cell epitope thereof.

In a preferred embodiment, the immunologically interactive molecule is an antibody that binds specifically to a polypeptide consisting of the amino acid of SEQ ID NO:1, or to the first forty two amino acids thereof.

Conventional methods can be used to prepare the immunologically interactive molecules. For example, by using a polypeptide of the present invention, polyclonal antisera or monoclonal antibodies can be made using standard methods. For example, a mammal, (e.g., a mouse, hamster, or rabbit) can be immunized with an immunogenic form of the polypeptide of the present invention which elicits an antibody response in the mammal. Techniques for conferring immunogenicity on a polypeptide include conjugation to carriers, or other techniques well known in the art. For example, the polypeptide can be administered in the presence of adjuvant or can be coupled to a carrier molecule, as known in the art, that enhances the immunogenicity of the polypeptide. The progress of immunization can be monitored by detection of antibody titres in plasma or serum. Standard ELISA or other immunoassay can be used with the immunogen as antigen to assess the levels of antibodies. Following immunization, antisera can be obtained and, for example, IgG molecules corresponding to the polyclonal antibodies can be isolated from the antisera.

To produce monoclonal antibodies, antibody producing cells (lymphocytes) can be harvested from an animal immunised with a peptide of the present invention and fused with myeloma cells by standard somatic cell fusion procedures, thus immortalizing these cells and yielding hybridoma cells. Such techniques are well known in the art, and include, for example, the hybridoma technique originally developed by Kohler and Milstein (1975), as well as other techniques such as the human B-cell hybridoma technique (Kozbor *et al.*, 1983), the EBV-hybridoma technique to produce human monoclonal antibodies (Cole *et al.*, 1985), and screening of combinatorial antibody libraries (Huse *et al.*, 1989). Hybridoma cells can be isolated and screened immunochemically for production of antibodies that are specifically reactive with the polypeptide and monoclonal antibodies isolated therefrom.

As with all immunogenic compositions for eliciting antibodies, the immunogenically effective amounts of the peptides of the invention must be determined empirically. Factors to be considered include the immunogenicity of the native peptide, whether or not the peptide will be complexed with or covalently attached to an adjuvant or carrier protein or other carrier, the route of administration for the composition, i.e.,

intravenous, intramuscular, subcutaneous, *etc.*, and the number of immunizing doses to be administered. Such factors are known in the vaccine art and it is well within the skill of immunologists to make such determinations without undue experimentation.

The term "antibody" as used herein, is intended to include fragments thereof which are also specifically reactive with a peptide that mimics or cross-reacts with a B-cell or T-cell epitope of the *Lawsonia intracellularis* SodC polypeptide set forth in SEQ ID NO:1. Antibodies can be fragmented using conventional techniques and the fragments screened for utility in the same manner as described above for whole antibodies. For example, F(ab')2 fragments can be generated by treating antibody with pepsin. The resulting F(ab')2 fragment can be treated to reduce disulfide bridges to produce Fab' fragments.

It is within the scope of this invention to include any secondary antibodies (monoclonal, polyclonal or fragments of antibodies), including anti-idiotypic antibodies, directed to the first mentioned antibodies discussed above. Both the first and second antibodies can be used in detection assays or a first antibody can be used with a commercially available anti-immunoglobulin antibody. An antibody as contemplated herein includes any antibody specific to any region of a peptide which mimics, or cross-reacts with a B-cell or T-cell epitope of the *Lawsonia intracellularis* SodC polypeptide set forth in SEQ ID NO:1 as hereinbefore described.

The antibodies described herein are useful for determining B-cell or T-cell epitopes of the amino acid sequence set forth in SEQ ID NO:1 such as, for example, by testing the ability of synthetic peptides to cross-react immunologically with said amino acid sequence or to elicit the production of antibodies which cross-react with said amino acid sequence. Using methods described herein, polyclonal antibodies, monoclonal antibodies or chimeric monoclonal antibodies can also be raised to peptides which mimic or cross-react with a B-cell or T-cell epitope of the *Lawsonia intracellularis* SodC polypeptide set forth in SEQ ID NO:1.

More particularly, the polyclonal, monoclonal or chimeric monoclonal antibodies can

be used to detect the peptides of the invention and/or any homologues, analogues or derivatives thereof, in various biological materials. For example, they can be used in an ELISA, radioimmunoassay, or histochemical test. In other words, the antibodies can be used to test for binding to a polypeptide of the invention or to a homologue, analogue or derivative thereof, in a biological sample to diagnose the presence of *Lawsonia intracellularis* therein.

Accordingly, a further aspect of the invention provides a method of diagnosing infection of an animal by *Lawsonia intracellularis* or a related microorganism thereto, said method comprising the steps of contacting a biological sample derived from said animal with an immunologically interactive molecule which is capable of binding to a peptide, oligopeptide or polypeptide comprising the amino acid sequence set forth in SEQ ID NO:1 or a homologue, analogue or derivative thereof, for a time and under conditions sufficient for an antigen:antibody complex to form, and detecting said complex formation. According to this embodiment of the present invention, the immunologically interactive molecule is preferably an antibody molecule prepared against the *Lawsonia intracellularis* SodC polypeptide set forth in SEQ ID NO:1 or an analogue or derivative thereof.

The biological sample is one which might contain a polypeptide having an amino acid sequence set forth in SEQ ID NO:1 or a homologue, analogue or derivative thereof, in particular a biological sample derived from a porcine or avian host of the pathogen *Lawsonia intracellularis* or a related microorganism thereto, and can include any appropriate tissue or fluid sample from the animal. Preferred biological samples are derived from the ileum, caecum, small intestine, large intestine, whole serum or lymph nodes of the porcine or avian host animal being tested. Alternatively or in addition the biological test sample may comprise faeces or a rectal swab derived from the animal.

To distinguish *L. intracellularis* from other microorganisms resident in the gut or other organ of an animal, the antibodies should not be prepared against highly-conserved epitopes of SodC such as those regions of at least 5 amino acids in length which are conserved between *L. intracellularis* and *E.coli* as set forth in Figure 1.

Conventional immunoassays can be used to perform this embodiment of the invention. A wide range of immunoassay techniques are available as can be seen by reference to US Patent Nos. 4,016,043, 4,424,279 and 4,018,653. These, of course, include both single-site and two-site or "sandwich" assays of the non-competitive types, as well as the traditional competitive binding assays. These assays also include direct binding of a labelled antibody to a target. It will be readily apparent to the skilled technician how to modify or optimise such assays to perform this embodiment of the present invention, and all such modifications and optimisations are encompassed by the present invention.

In one alternative embodiment, the present invention contemplates a method of identifying whether or not an animal has suffered from a past infection, or is currently infected with *Lawsonia intracellularis* or a related microorganism thereto, said method comprising contacting blood or serum derived from said animal with the immunogenic polypeptide of the invention for a time and under conditions sufficient for an antigen:antibody complex to form, and detecting said complex formation. This embodiment differs from the embodiment described *supra* in that it relies upon the detection of circulating antibodies against *Lawsonia intracellularis* or related organism in the animals blood or serum which are present as a consequence of a past or present infection by this pathogen. However, it will be apparent to those skilled in the art that the principle of the assay format is the same. As with other embodiments of the invention referred to *supra*, conventional immunoassays can be used. Persons skilled in the art will readily be capable of varying known immunoassay formats to perform the present embodiment. This embodiment of the invention can also utilise derivatives of blood and serum which comprise immunologically interactive molecules such as, for example, partially-purified IgG or IgM fractions and buffy coat samples, amongst others. The preparation of such fractions will also be known to those skilled in the art.

A further aspect of the present invention provides an isolated nucleic acid molecule which comprises a sequence of nucleotides which encodes, or is complementary to a nucleic acid molecule which encodes, a peptide, oligopeptide or polypeptide selected from the following:

(i) a peptide, oligopeptide or polypeptide which comprises an amino acid sequence having at least about 70% identical overall to the amino acid sequence set forth in SEQ ID NO:1;

(ii) a peptide, oligopeptide or polypeptide which comprises an amino acid sequence having at least about 50% identity to amino acid residues from about position 1 to about position 42 of SEQ ID NO:1;

(iii) a peptide comprising at least 5 contiguous amino acids derived from SEQ ID NO:1; or

(iv) a homologue, analogue or derivative of (i) or (ii) which mimics a B-cell or T-cell epitope of *Lawsonia spp*.

In a preferred embodiment, the present invention provides an isolated nucleic acid molecule comprising a sequence of nucleotides which encodes, or is complementary to a nucleic acid molecule which encodes, a polypeptide immunogen which comprises, mimics or cross-reacts with a B-cell or T-cell epitope of the *Lawsonia intracellularis* SodC polypeptide set forth in SEQ ID NO:1.

In a particularly preferred embodiment, the present invention provides an isolated nucleic acid molecule comprising a sequence of nucleotides encoding the *L. intracellularis* SodC polypeptide having an amino acid sequence set forth in SEQ ID NO: 1 or encoding about the first forty-two amino acid residues thereof.

It is within the scope of the invention to encompass polymeric forms of the immunogenic polypeptide described herein, such as aggregates of the amino acid sequence set forth in SEQ ID NO:1 or a homologue, analogue or derivative thereof or, alternatively, as polypeptides comprising repeats of the amino acid sequence set forth in SEQ ID NO:1 or a homologue, analogue or derivative thereof. The present invention extends further to nucleic acid molecules encoding such polymeric forms. thereof.

Alternatively or in addition, the isolated nucleic acid molecule of the invention further

comprises a sequence of nucleotides which has at least about 70% overall sequence identity to the nucleotide sequence set forth in SEQ ID NO:2 or to a complementary nucleotide sequence thereof. More preferably, the percentage sequence identity to SEQ ID NO:2 or to a complementary nucleotide sequence thereto is at least about 80%. Still more preferably, the percentage sequence identity is at least about 90%. Yet still more preferably, the percentage sequence identity is at least about 95%.

In a preferred embodiment, the nucleic acid molecule comprises the nucleotide sequence set forth in SEQ ID NO:2, or the SodC-encoding nucleotide sequence present in pALK14 (ATCC 207155), or a degenerate variant thereof, and complements thereof.

In determining whether or not two nucleotide sequences fall within these percentage limits, those skilled in the art will be aware that it is necessary to conduct a side-by-side comparison or multiple alignment of sequences. In such comparisons or alignments, differences may arise in the positioning of non-identical residues, depending upon the algorithm used to perform the alignment. In the present context, reference to a percentage identity between two or more nucleotide sequences shall be taken to refer to the number of identical residues between said sequences as determined using any standard algorithm known to those skilled in the art. For example, nucleotide sequences may be aligned and their identity calculated using the BESTFIT programme or other appropriate programme of the Computer Genetics Group, Inc., University Research Park, Madison, Wisconsin, United States of America (Devereaux *et al*, 1984).

Alternatively or in addition, the isolated nucleic acid molecule of the invention is capable of hybridising under at least low stringency conditions to the nucleotide sequence set forth in SEQ ID NO:2 or to a complementary nucleotide sequence thereto or to a nucleic acid fragment comprising at least about 20 contiguous nucleotides in length derived from the sequence set forth in SEQ ID NO:2 or to a complementary nucleotide sequence thereto.

Preferably, said nucleic acid molecule is capable of hybridising under at least moderate stringency conditions, and even more preferably under high stringency conditions.

For the purposes of defining the level of stringency, a low stringency is defined herein as being a hybridisation and/or a wash carried out in 6xSSC buffer, 0.1% (w/v) SDS at 28°C. A moderate stringency is defined herein as being a hybridisation and/or wash carried out in 2xSSC buffer, 0.1% (w/v) SDS at a temperature in the range 45°C to 65°C. A high stringency is defined herein as being a hybridisation and/or wash carried out in 0.1xSSC buffer, 0.1% (w/v) SDS at a temperature of at least 65°C.

Generally, the stringency is increased by reducing the concentration of SSC buffer, and/or increasing the concentration of SDS and/or increasing the temperature of the hybridisation and/or wash. Those skilled in the art will be aware that the conditions for hybridisation and/or washing may vary depending upon the nature of the hybridisation membrane or the type of hybridisation probe used. Conditions for hybridisations and washes are well understood by one normally skilled in the art. For the purposes of clarification of the parameters affecting hybridisation between nucleic acid molecules, reference is found in pages 2.10.8 to 2.10.16. of Ausubel *et al.* (1987), which is herein incorporated by reference.

The present invention clearly encompasses genetic constructs comprising the subject nucleic acid molecule in an expressible format suitable for the preparation of a recombinant immunogenic polypeptide of the present invention, such as for use in recombinant univalent or polyvalent recombinant vaccines.

In such cases, the nucleic acid molecule will be operably connected to a promoter sequence which can thereby regulate expression of said nucleic acid molecule in a prokaryotic or eukaryotic cell as described *supra*.

The genetic construct optionally further comprises a terminator sequence. The term "terminator" refers to a DNA sequence at the end of a transcriptional unit which signals termination of transcription. A "terminator" is a nucleotide sequence, generally located

within the 3'-non-translated region of a gene or mRNA, comprising a polyadenylation signal to facilitate the post-transcriptional addition of a polyadenylate sequence to the 3'-end of a primary mRNA transcript. Terminator sequences may be isolated from the genetic sequences of bacteria, fungi, viruses, animals and/or plants. Terminators active in animal cells are known and described in the literature.

In a preferred embodiment, the genetic construct can be a cloning or expression vector, as known in the art, such as a plasmid, cosmid, or phage, comprising a nucleic acid molecule of the present invention, and host cells transformed or transfected therewith. In a non-limiting embodiment, the vector is plasmid pALK14 (ATCC Accession No. 207155).

The genetic constructs of the present invention are particularly useful for producing the proteinaceous immunogenic component of the vaccine composition described herein or for use in a DNA vaccine.

A range of genetic diagnostic assays to detect infection of an animal by *Lawsonia intracellularis* or a related microorganism can be employed using the nucleic acid molecule described herein such as, for example, assays based upon the polymerase chain reaction (PCR) and nucleic acid hybridisation. All such assays are contemplated in the present invention.

Accordingly, a still further aspect of the invention provides a diagnostic method of detecting *Lawsonia intracellularis* or related microorganism in a biological sample derived from an animal subject, said method comprising the steps of hybridising one or more probes or primers derived from the nucleotide sequence set forth in SEQ ID NO:2 or a complementary nucleotide sequence thereto or a homologue, analogue or derivative thereof, to a DNA or RNA molecule present in said sample and then detecting said hybridisation using a detection means.

As used herein, the term "probe" refers to a nucleic acid molecule which is derived from the nucleotide sequence set forth in SEQ ID NO:2 and which is capable of being

used in the detection thereof. Probes may comprise DNA (single-stranded or double-stranded) or RNA (i.e., riboprobes) or analogues thereof.

The term "primer" refers to a probe as hereinbefore defined which is further capable of being used to amplify a nucleotide sequence from *Lawsonia intracellularis* or a related microorganism thereto, in a PCR.

Preferred probes and primers include fragments of the nucleotide sequence set forth in SEQ ID NO:2 and synthetic single-stranded DNA or RNA molecules of at least about 15 nucleotides in length derived from the sequence set forth in SEQ ID NO:2 or a complementary nucleotide sequence thereto.

Preferably, probes and primers according to this embodiment will comprise at least about 20 contiguous nucleotides derived from SEQ ID NO:2 or a complementary sequence thereto, even more preferably at least about 25 contiguous nucleotides, still even more preferably at least about 50 contiguous nucleotides and even more preferably at least about 100 nucleotides to about 500 nucleotides derived from the sequence set forth in SEQ ID NO:2 or a complement thereof. Probes and primers comprising the full-length of SEQ ID NO:2 or a complementary nucleotide sequence thereto are also encompassed by the present invention.

For the present purpose, "homologues" of a nucleotide sequence shall be taken to refer to an isolated nucleic acid molecule which encodes a polypeptide that is functionally equivalent to the polypeptide encoded by the nucleic acid molecule of the present invention or to a polypeptide which is a homologue, analogue or derivative of SEQ ID NO:1, notwithstanding the occurrence within said sequence, of one or more nucleotide substitutions, insertions, deletions, or rearrangements.

"Analogues" of a nucleotide sequence set forth herein shall be taken to refer to an isolated nucleic acid molecule which encodes a functionally-equivalent polypeptide to the polypeptide encoded by the nucleic acid molecule of the present invention or a homologue, analogue or derivative of a polypeptide having the amino acid sequence

of SEQ ID NO:1, notwithstanding the occurrence of any non-nucleotide constituents not normally present in said isolated nucleic acid molecule such as, for example, carbohydrates, radiochemicals including radio nucleotides, reporter molecules such as, but not limited to biotin, DIG, alkaline phosphatase or horseradish peroxidase, amongst others.

"Derivatives" of a nucleotide sequence set forth herein shall be taken to refer to any isolated nucleic acid molecule which contains at least about 50% nucleotide sequence identity to 15 or more contiguous nucleotides present in the nucleotide sequence set forth in SEQ ID NO:2 or a complementary nucleotide sequence thereto. Generally, the nucleotide sequence of the present invention may be subjected to mutagenesis to produce single or multiple nucleotide substitutions, deletions and/or insertions. Nucleotide insertional derivatives of the nucleotide sequence of the present invention include 5′ and 3′ terminal fusions as well as intra-sequence insertions of single or multiple nucleotides or nucleotide analogues. Insertional nucleotide sequence variants are those in which one or more nucleotides or nucleotide analogues are introduced into a predetermined site in the nucleotide sequence of said sequence, although random insertion is also possible with suitable screening of the resulting product being performed. Deletional nucleotide sequence variants are characterised by the removal of one or more nucleotides from the nucleotide sequence. Substitutional nucleotide sequence variants are those in which at least one nucleotide in the sequence has been removed and a different nucleotide or nucleotide analogue inserted in its place. In a preferred embodiment, such substitutions are selected based on the degeneracy of the genetic code, as known in the art, with the resulting substitutional variant encoding the amino acid sequence of SEQ ID NO:1 or at least about the first 42 amino acids thereof.

Probes or primers can comprise inosine, adenine, guanine, thymidine, cytidine or uracil residues or functional analogues or derivatives thereof that are capable of being incorporated into a polynucleotide molecule, provided that the resulting probe or primer is capable of hybridising under at least low stringency conditions to SEQ ID NO:2 or to a complementary nucleotide sequence thereof, or is at least about 70% identical to

SEQ ID NO:2 or to a complementary nucleotide sequence thereof.

The biological sample according to this aspect of the invention includes any organ, tissue, cell or exudate which contains or is likely to contain *Lawsonia intracellularis* or a nucleic acid derived therefrom. A biological sample can be prepared in a suitable solution such as, for example, an extraction buffer or suspension buffer. The present invention extends to the testing of biological solutions thus prepared, the only requirement being that said solution at least comprises a biological sample as described herein.

The diagnostic assay of the present invention is useful for the detection of *Lawsonia intracellularis* or a microorganism which is related thereto which expresses the SodC polypeptide of the present invention or a SodC-like polypeptide.

The present invention clearly contemplates diagnostic assays which are capable of both genus-specific and species-specific detection. Accordingly, in one embodiment, the probe or primer, or a homologue, analogue or derivative thereof, comprises DNA capable of being used to detect multiple *Lawsonia spp.* In an alternative embodiment, the probe or primer or a homologue, analogue or derivative thereof comprises DNA capable of being used to distinguish *Lawsonia intracellularis* from related microorganisms.

Less-highly conserved regions within SEQ ID NO:2, such as those encoding about amino acid residues from about position 1 to about position 42 of the *Lawsonia intracellularis* SodC polypeptide set forth in SEQ ID NO:1, are particularly useful as species-specific probes and/or primers for the detection of *L. intracellularis* and very closely related species.

Furthermore, the diagnostic assays described herein can be adapted to a genus-specific or species-specific assay by varying the stringency of the hybridisation step. Accordingly, a low stringency hybridisation can be used to detect several different species of *Lawsonia* in one or more biological samples being assayed, while a high

stringency hybridisation can be used to distinguish *Lawsonia intracellularis* from such other species.

The detection means according to this aspect of the invention may be any nucleic acid-based detection means such as, for example, nucleic acid hybridisation techniques or paper chromatography hybridisation assay (PACHA), or an amplification reaction such as PCR, or nucleic acid sequence-based amplification (NASBA) system. The invention further encompasses the use of different assay formats of said nucleic acid-based detection means, including restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), single-strand chain polymorphism (SSCP), amplification and mismatch detection (AMD), interspersed repetitive sequence polymerase chain reaction (IRS-PCR), inverse polymerase chain reaction (iPCR), *in situ* polymerase chain reaction and reverse transcription polymerase chain reaction (RT-PCR), amongst others.

Where the detection means is a nucleic acid hybridisation technique, the probe can be labelled with a reporter molecule capable of producing an identifiable signal (e.g., a radioisotope such as ^{32}P or ^{35}S, or a biotinylated molecule). According to this embodiment, those skilled in the art will be aware that the detection of said reporter molecule provides for identification of the probe and that, following the hybridisation reaction, the detection of the corresponding nucleotide sequences in the biological sample is facilitated. Additional probes can be used to confirm the assay results obtained using a single probe.

A variation of the nucleic acid hybridisation technique contemplated by the present invention is the paper chromatography hybridisation assay (PACHA) described by Reinhartz *et al.* (1993) and equivalents thereof, wherein a target nucleic acid molecule is labelled with a reporter molecule such as biotin, applied to one end of a nitrocellulose or nylon membrane filter strip and subjected to chromatography under the action of capillary or other forces (e.g., an electric field) for a time and under conditions sufficient to promote migration of said target nucleic acid along the length of said membrane to a zone at which a DNA probe is immobilised thereto such as, for

example, in the middle region. According to this detection format, labelled target nucleic acid comprising the *Lawsonia spp.* nucleotide sequences complementary to the probe will hybridise thereto and become immobilised in that region of the membrane to which the probe is bound. Non-complementary sequences to the probe will diffuse past the site at which the probe is bound. The target nucleic acid may comprise a crude or partially-pure extract of DNA or RNA or, alternatively, an amplified or purified DNA. Additional variations of this detection means which utilise the nucleotide sequences described herein are clearly encompassed by the present invention.

Wherein the detection means is a RFLP, nucleic acid derived from the biological sample, in particular DNA, is digested with one or more restriction endonuclease enzymes and the digested DNA is subjected to electrophoresis, transferred to a solid support such as, for example, a nylon or nitrocellulose membrane, and hybridised to a probe optionally labelled with a reporter molecule as hereinbefore defined. According to this embodiment, a specific pattern of DNA fragments is displayed on the support, wherein said pattern is preferably specific for a particular *Lawsonia* species to enable the user to distinguish between different species of the bacterium.

Wherein the detection means is an amplification reaction such as, for example a polymerase chain reaction or a nucleic acid sequence-based amplification (NASBA) system or a variant thereof same, one or more nucleic acid primer molecules of at least 15 contiguous nucleotides in length derivable from SEQ ID NO:2 or its complementary nucleotide sequence, or a homologue, analogue or derivative thereof, is hybridised to nucleic acid derived from a biological sample, and nucleic acid copies of the SodC-encoding genetic sequences in said sample, or a part or fragment thereof, are enzymically-amplified.

Those skilled in the art will be aware that there must be a sufficiently high percentage of nucleotide sequence identity between the primers and the sequences in the biological sample template molecule to which they hybridise (i.e., the "template molecule"). As stated previously, the stringency conditions can be varied to promote

hybridisation.

Preferably, each primer is at least about 95% identical to a region of SEQ ID NO:2 or its complementary nucleotide sequence in the template molecule to which it hybridises.

Those skilled in the art will also be aware that, in one format, PCR provides for the hybridisation of non-complementary primers to different strands of the template molecule, such that the hybridised primers are positioned to facilitate the 5'→ 3' synthesis of nucleic acid in the intervening region, under the control of a thermostable DNA polymerase enzyme. As a consequence, PCR provides an advantage over other detection means in so far as the nucleotide sequence in the region between the hybridised primers may be unknown and unrelated to any known nucleotide sequence.

In an alternative embodiment, wherein the detection means is AFLP, the primers are selected such that, when nucleic acid derived from the biological sample, in particular DNA, is amplified, different length amplification products are produced from different *Lawsonia spp.* The amplification products can be subjected to electrophoresis, transferred to a solid support such as, for example, a nylon or nitrocellulose membrane, and hybridised to a probe optionally labelled with a reporter molecule, as hereinbefore described. According to this embodiment, a specific pattern of amplified DNA fragments is displayed on the support, said pattern optionally specific for a particular *Lawsonia ssp.*, to enable the user to distinguish between different species of the bacterium in much the same way as for RFLP analysis.

The technique of AMD facilitates, not only the detection of *Lawsonia spp.* DNA in a biological sample, but also the determination of nucleotide sequence variants which differ from the primers and probes used in the assay format. Wherein the detection means is AMD, the probe is end-labelled with a suitable reporter molecule and mixed with an excess of the amplified template molecule. The mixtures are subsequently denatured and allowed to renature to form nucleic acid "probe:template hybrid molecules" or "hybrids", such that any nucleotide sequence variation between the probe and the temple molecule to which it is hybridised will disrupt base-pairing in the

hybrids. These regions of mismatch are sensitive to specific chemical modification using hydroxylamine (mismatched cytosine residues) or osmium tetroxide (mismatched thymidine residues), allowing subsequent cleavage of the modified site using piperidine. The cleaved nucleic acid may be analysed using denaturing polyacrylamide gel electrophoresis, followed by standard nucleic acid hybridisation as described *supra*, to detect the *Lawsonia*-derived nucleotide sequences. Those skilled in the art will be aware of the means of end-labelling a genetic probe according to the performance of the invention described in this embodiment.

According to this embodiment, the use of a single end-labelled probe allows unequivocal localisation of the sequence variation. The distance between the point(s) of sequence variation and the end-label is represented by the size of the cleavage product.

In an alternative embodiment of AMD, the probe is labelled at both ends with a reporter molecule, to facilitate the simultaneous analysis of both DNA strands.

Wherein the detection means is RT-PCR, the nucleic acid sample comprises an RNA molecule which is a transcription product of *Lawsonia*-derived DNA or a homologue, analogue or derivative thereof. As a consequence, this assay format is particularly useful when it is desirable to determine expression of one or more *Lawsonia* genes. According to this embodiment, the RNA sample is reverse-transcribed to produce the complementary single-stranded DNA which is subsequently amplified using standard procedures.

Variations of the embodiments described herein are described in detail by McPherson *et al.* (1991).

The present invention clearly extends to the use of any and all detection means referred to *supra* for the purposes of diagnosing *Lawsonia spp.* and in particular *Lawsonia intracellularis* infection in animal.

The amplification reaction detection means described *supra* can be further coupled to a classical hybridisation reaction detection means to further enhance sensitivity and specificity of the inventive method, such as by hybridising the amplified DNA with a probe which is different from any of the primers used in the amplification reaction.

Similarly, the hybridisation reaction detection means described *supra* can be further coupled to a second hybridisation step employing a probe which is different from the probe used in the first hybridisation reaction.

A further aspect of the invention provides an isolated probe or primer derived from SEQ ID NO:2 or a complementary nucleotide sequence thereto.

The present invention is further described by the following non-limiting examples.

EXAMPLE 1

SOURCES OF PIG TISSUE

Infected Pig Intestines

Sections of grossly thickened ilea were taken from pigs naturally or experimentally affected by PPE. The presence of *L. intracellularis* bacteria in the ilea was confirmed using immunofluorescent staining with specific monoclonal antibodies (McOrist *et al*, 1987). An example of a suitable antibody is monoclonal antibody IG4 available from the University of Edinburgh, UK.

EXAMPLE 2

ISOLATION OF *Lawsonia intracellularis* BACTERIA FROM THE INFECTED PIG ILEUM

Lawsonia intracellularis bacteria were extracted directly from lesions of PPE in pigs by filtration and further purified over a Percoll (Pharmacia, Uppsala, Sweden) gradient as

follows. Infected ilea were collected from pigs and the presence of *L. intracellularis* was confirmed histologically before storage at -80°C. Sections of ileum were thawed and approximately 8g of infected mucosa were scraped from the intestinal wall. The mucosa was homogenised with 40 ml sterile phosphate buffered saline (PBS) on half speed for 10 seconds using a Sorvall omnimixer. This suspension was centrifuged at 2000 *xg* for 4 minutes. The supernatant was discarded and the cell pellet was resuspended in 40 ml PBS and re centrifuged. This washing step was repeated twice. The cell pellet was then resuspended in 20 ml PBS and homogenised at full speed for one minute to release *L. intracellularis* bacteria.

This homogenate was centrifuged at 1000 *xg* for 4 minutes giving a pellet containing a crude mixture of homogenised epithelial cells and intestinal bacteria. The supernatant was filtered using filters with pore sized 3 μm, 1.2 μm and 0.8 μm (Millipore Corporation, MA, USA). The filtrate was centrifuged at 8000 *xg* for 30 minutes, resulting in a small pellet of *L. intracellularis* bacteria. The *L. intracellularis* bacteria were further purified using a 45% self forming Percoll gradient as follows: 2 mls of the bacterial preparation was mixed by inversion into 30 mls of a 45% self forming Percoll (Pharmacia LKB, Uppsala, Sweden) gradient (45% v/v of Percoll, 150 mM NaCl). The gradients were centrifuged in a Sorval centrifuge using the SS34 rotor, at 20,000 rpm for 30 minutes at 4°C. Usually a number of bands form within the gradient. The band (usually located approx. 10-20 mm from the base of the tube) containing the *L. intracellularis* bacteria was collected and the volume made up to 16 mls with PBS. The solution was then centrifuged for 15 minutes at 8000rpm. The resultant pellet was washed with PBS before being resuspended in a final volume of approximately one ml.

EXAMPLE 3

PURIFICATION OF *Lawsonia intracellularis* GENOMIC DNA

Genomic DNA was extracted from Percoll-gradient purified *Lawsonia intracellularis* bacteria recovered from infected pig ilea scrapings (Example 2) by the methods described by Anderson *et al* (1984) and Sambrook *et al* (1989).

Briefly, the *L. intracellularis* cells were pelleted by centrifugation at 14,000 x g at 4°C for 15 min. The cells were resuspended in 10 ml of TE buffer (1 mM Tris-HCl, 0.1 mM EDTA, pH 8.0) and centrifuged as before. The pellet was then resuspended in 4 ml of TE buffer containing 4 mg/ml lysozyme (Sigma Chemical Co.) and incubated at 37°C for 20 min. SDS and proteinase K (Promega, WI, USA) were added to final concentrations of 1% (w/v) and 200 μg/ml, respectively, and incubation was continued at 45°C for 4 hours. The lysate was then extracted with an equal volume of phenol, phenol:chloroform (1:1) and chloroform, respectively, and the nucleic acids were recovered from the supernatant by ethanol precipitation. The pellet was gently dissolved in TE, treated with RnaseA (Promega, WI, USA) at 37°C for 30 min and then digested with proteinase K in the presence of 0.5% (w/v) SDS for 1 h at 50°C. After another round of phenol:chloroform (1:1) and ethanol precipitation, the purified DNA was dissolved in TE. The DNA was then stored at 4°C.

EXAMPLE 4

IMMUNOSCREENING OF A *L. intracellularis* LIBRARY USING EXPERIMENTAL SERA FROM VACCINATED PIGS

The genomic DNA from Example 3 was partially digested with the restriction endonuclease *Sau*3A (Promega) and ligated into Lambda ZAP Express (Stratagene, CA, USA). The lambda library was plated on a lawn of *E. coli* XLI-Blue cells at a density of 1,000 phage forming units (pfu) per 150 mm L-broth agar plate. The library was screened using the method described in the Protoblot Technical Manual (Promega, WI, USA). The filters were blocked in blocking buffer (10 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.05% Tween 20 and 5% blotto) prior to screening with sera from the pigs Y12 and/or 395. The pigs Y12 and 395 had previously been immunised with formalin-killed *L. intracellularis* and heat-killed *L. intracellularis*, respectively, as described in International Patent Application No. PCT/AU96/00767. Positive plaques identified in the primary screen were picked, replated at a lower density and rescreened with either or both sera until an individual positive plaque was identified. Plasmid DNA from the positive lambda phage clone was isolated by *in vivo* excision, as recommended by the manufacturer (Stratagene, CA, USA). This clone, p98LI50,

contained the *sodC* gene of *L. intracellularis*.

EXAMPLE 5

ANALYSIS OF *L. intracellularis* EXPRESSING PHAGE CLONES

Phagemid DNA from positive λZAP Express phage clones was isolated by *in vivo* excision, by the conditions recommended by the manufacturer (Stratagene).

Plasmid DNA for restriction analysis was extracted by alkaline-lysis, as described by Sambrook *et al* (1989), and for automated sequencing, using the High Pure Plasmid Kit, as recommended by the manufacturer (Boehringer Mannheim, Mannheim, Germany).

The nucleotide sequence of the *sodC* gene of *L. intracellularis* was obtained from the clone p98LI50 identified in the preceding Example. DNA sequencing was performed by the Dye-terminator method of automated sequencing (ABI Biosystems, CA, USA).The nucleotide sequence of the complete coding region of the *sodC* gene is set out in SEQ ID NO: 2.

EXAMPLE 6

IDENTIFICATION OF *L. Intracellularis* COMPONENTS

Sequence similarity of the DNA molecules encoding putative vaccine candidates identified from Example 4 and 5, was identified using the BLAST algorithm(Gish and States, 1993). Amino acid residues 43 to 57 and 62 to 64 of the *Lawsonia intracellularis* SodC sequence set froth in SEQ ID NO:1 have very high sequence similarity to the *Escherichia coli* SodC polypeptides (Figure 1). Unique regions, in particular residues 1 to 42 of the *Lawsonia intracellularis* SodC polypeptide are apparent from a comparison of the amino acid sequence of this polypeptide to those from other microorganisms (Figure 1).

EXAMPLE 7

EXPRESSION OF THE FULL-LENGTH SodC PROTEIN

The primary sequence of the native SodC protein appears to match the sequence requirements for both a standard secretory signal peptide cleavage site and a prokaryotic lipoprotein cleavage site. Both cleavage sites are located at Ala26-Cys27 of the amino acid sequence set forth in SEQ ID NO:1.

The expression of certain proteins can often be improved by fusing a short leader peptide or a "protective peptide" to the N-terminus of the target protein. This protective peptide protects recombinant proteins from proteolysis (Sung *et al.*, 1986; 1987 and United States Patent No. 5,460,954).

To express the SodC protein, the nucleotide DNA sequence encoding the putative signal peptide of the SodC protein was removed from the full-length *SodC* gene (SEQ ID NO:2), to yield a signal sequence-deficient SodC polypeptide-encoding sequence, designated SS(-)SodC. Second, a nucleotide sequence encoding the protective peptide leader amino acid sequence MGTTTTTTSL (PP; SEQ ID NO:3)was fused to the 5′-end of SS(-)SodC, to yield PP-SS(-)SodC. Cloning details are presented in Example 8 below.

EXAMPLE 8

AMPLIFICATION OF *SodC*

Template DNA for *SodC* was plasmid p98LI50. Plasmid p98LI50 was excised from Lambda ZAP Express (Stratagene Cloning Systems, La Jolla, California) and is a pBK-CMV derivative, which was identified by screening a *L. intracellularis* genomic lambda libraries with α-*L. intracellularis* pig antisera, as described in Example 4.

The PCR amplifications consisted of 0.1 ng of plasmid template, 1 μM each of the forward primer (RA167:5′: GGCCATGGGTACCACCACCACCACCACCTCTCTGTC TGTTACTTCAGAAGTCCATATG 3′; SEQ ID NO: 4); and the reverse primer (RA175: 5′ GGCTCTAGAGGTATATAAATATAAAGAGGTATG 3′; SEQ ID NO: 5); 7.5 units KlenTaqI polymerase (Ab Peptides, Inc., St. Louis, Missouri), 0.075 units *Pfu*

polymerase (Stratagene Cloning Systems, La Jolla, California) 1 x PC2 (KlenTaqI) buffer and 0.2 mM dNTPs in a 50 μl volume. PCR was carried out in 4 stages: (i) 95°C for 5 min (5'); (ii) 94°C for 1 min, 58°C for 30 seconds, 72°C for 1.5 min, x 33 cycles; (iii) 72°C for 10 min, (iv) hold at 4°C.

The PCR fragment containing the *SodC* gene of *L. intracellularis* was subcloned into pCR2.1-TOPO (Invitrogen Corp., Carlsbad, CA) and designated pALK14. The pCR2.1-TOPO intermediate plasmid was digested with *Nco*I and *Eco*RI and the 0.6 kb fragment excised therefrom was gel-purified and sub-cloned into *Nco*I- *Eco*RI-digested pET28b, to produce the PP-SS(-)SodC expression plasmid, pRL032. In this plasmid, the ATG start codon of PP-SS(-)SodC is directly downstream from the ribosome-binding site. The expression of the PP-SS(-)SodC protein from this plasmid is under control of the T7 promoter, which is inducible by IPTG. The plasmid was introduced into *E. coli* BL21 (DE3) cells for expression of the modified SodC protein.

MICROORGANISM DEPOSITS

The plasmid pALK14 was deposited with the American Type Culture Collection (ATCC) at 10801 University Boulevard, Manassas, VA 20110, USA on 11th March, 1999 and was assigned ATCC Accession No.207155.

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WE CLAIM:

1. An isolated or recombinant immunogenic polypeptide which comprises, mimics or cross-reacts with a B-cell or T-cell epitope of a *Lawsonia spp.* SodC polypeptide.

2. The isolated or recombinant immunogenic polypeptide of claim 1 capable of eliciting the production of antibodies against *Lawsonia spp.* when administered to an avian or porcine animal.

3. The isolated or recombinant immunogenic polypeptide of claim 1 capable of conferring a protective immune response against *Lawsonia spp.* when administered to an avian or porcine animal.

4. The isolated or recombinant immunogenic polypeptide of claim 2 wherein the *Lawsonia spp.* is *L. intracellularis.*

5. The isolated or recombinant immunogenic polypeptide of claim 3 wherein the *Lawsonia spp.* is *L. intracellularis.*

6. An isolated or recombinant immunogenic polypeptide selected from the following:

(i) a peptide, oligopeptide or polypeptide which comprises an amino acid sequence which has at least about 70% sequence identity overall to the amino acid sequence set forth in SEQ ID NO: 1;

(ii) a peptide, oligopeptide or polypeptide which comprises an amino acid sequence having at least about 50% sequence identity overall to amino acid residues 1 to 42 of SEQ ID NO: 1; or

(iii) a homologue, analogue or derivative of (i) or (ii) which mimics a B-cell or T-cell epitope of a *Lawsonia spp*. SodC polypeptide.

7. The isolated or recombinant immunogenic polypeptide of claim 6 capable of eliciting the production of antibodies against *Lawsonia spp.* in a porcine or avian

animal.

8. The isolated or recombinant immunogenic polypeptide of claim 7 capable of conferring a protective immune response against *Lawsonia spp.* in a porcine or avian animal.

9. The isolated or recombinant immunogenic polypeptide of claim 8, capable of inducing humoral immunity against *Lawsonia spp.* in a porcine or avian animal.

10. The isolated or recombinant immunogenic polypeptide of claim 9, capable of inducing humoral immunity against *Lawsonia spp.* in a porcine animal.

11. The isolated or recombinant immunogenic polypeptide of claim 8 wherein the *Lawsonia spp.* is *L. intracellularis*.

12. The isolated or recombinant immunogenic polypeptide of claim 10 wherein the *Lawsonia spp.* is *L. intracellularis*.

13. The isolated or recombinant immunogenic polypeptide of claim 6 that comprises the amino acid sequence set forth in SEQ ID NO: 1 or the amino acid sequence encoded by the SodC-encoding nucleotide sequence of pALK14 (ATCC 207155) and is capable of eliciting the production of antibodies against *Lawsonia intracellularis* when administered to an avian or porcine animal.

14. The isolated or recombinant immunogenic polypeptide of claim 13 that consists essentially of the amino acid sequence of SEQ ID NO: 1 or the amino acid sequence encoded by the SodC-encoding nucleotide sequence of pALK14 (ATCC 207155) .

15. The isolated or recombinant immunogenic polypeptide of claim 13 or 14 capable of inducing a protective immune response against *Lawsonia intracellularis* in a porcine or avian animal.

16. The isolated or recombinant immunogenic polypeptide of claim 15 capable of inducing a protective immune response against *Lawsonia intracellularis* in a porcine animal.

17. The isolated or recombinant immunogenic polypeptide of claim 6 that comprises amino acid residues about 1 to about 42 of SEQ ID NO: 1 and is capable of eliciting the production of antibodies against *Lawsonia intracellularis* when administered to an avian or porcine animal.

18. The isolated or recombinant immunogenic polypeptide of claim 17 that consists essentially of about amino acid 1 to about amino acid 42 of SEQ ID NO: 1.

19. The isolated or recombinant immunogenic polypeptide of claim 17 capable of inducing a protective immune response against *Lawsonia intracellularis* in a porcine or avian animal.

20. The isolated or recombinant immunogenic polypeptide of claim 19 capable of inducing a protective immune response against *Lawsonia intracellularis* in a porcine animal.

21. A vaccine composition for the prophylaxis or treatment of infection of an animal by *Lawsonia spp.*, said vaccine composition comprising an effective amount of an immunogenic component which comprises an isolated or recombinant polypeptide having at least about 70% sequence identity overall to the amino acid sequence set forth in SEQ ID NO: 1 or at least about 50% sequence identity overall to amino acid residues 1 to 42 of SEQ ID NO: 1 or an immunogenic homologue, analogue or derivative thereof which is immunologically cross-reactive with *Lawsonia intracellularis;* and one or more carriers, diluents or adjuvants suitable for veterinary or pharmaceutical use.

22. The vaccine composition according to claim 21 wherein the *Lawsonia spp.* is *L. intracellularis*.

23. The vaccine composition according to claim 22 wherein the immunogenic component comprises an isolated or recombinant polypeptide that comprises the amino acid sequence set forth in SEQ ID NO: 1 or the amino acid sequence encoded by the SodC-encoding nucleotide sequence of pALK14 (ATCC 207155) .

24. The vaccine composition of claim 23, wherein the immunogenic component consists essentially of the amino acid sequence of SEQ ID NO: 1.

25. The vaccine composition according to claim 22 wherein the immunogenic component comprises an isolated or recombinant polypeptide that comprises about amino acid residue 1 to about amino acid residue 42 of SEQ ID NO: 1.

26. The vaccine composition of claim 25, wherein the immunogenic component consists essentially of about amino acid 1 to about amino acid 42 of SEQ ID NO: 1.

27. A combination vaccine composition for the prophylaxis or treatment of infection of an animal by *Lawsonia spp.*, said vaccine composition comprising:

(i) a first immunogenic component which comprises an isolated or recombinant polypeptide having at least about 70% sequence identity overall to the amino acid sequence set forth in SEQ ID NO: 1 or at least about 50% sequence identity overall to amino acid residues 1 to 42 of SEQ ID NO: 1 or an immunogenic homologue, analogue or derivative thereof which is immunologically cross-reactive with *Lawsonia intracellularis;*

(ii) a second immunogenic component comprising an antigenic *L. intracellularis* peptide, polypeptide or protein; and

(iii) one or more carriers, diluents or adjuvants suitable for veterinary or pharmaceutical use.

28. A vaccine vector that comprises, in an expressible form, an isolated nucleic acid molecule having a nucleotide sequence that encodes an isolated or recombinant immunogenic polypeptide which comprises the amino acid sequence set forth in SEQ ID NO: 1, such that said immunogenic polypeptide is expressible at a level sufficient

to confer immunity against *Lawsonia spp.*, when administered to a porcine or avian animal.

29. The vaccine vector of claim 28 wherein the immunogenic polypeptide is expressed using the steps of:

(i) placing an isolated nucleic acid molecule which comprises the nucleotide sequence set forth in SEQ ID NO: 2 or degenerate variant, a homologue, analogue or derivative thereof which has at least about 70% sequence identity thereto, in operable connectin with a promoter sequence;

(ii) introducing the isolated nucleic acid molecule and promoter sequence of step (a) into the vaccine vector; and

(iii) incubating, growing, or propagating the vaccine vector for a time and under conditions sufficient for expression of the immunogenic polypeptide encoded by said nucleic acid molecule to occur.

30. The vaccine vector of claim 28 wherein the *Lawsonia spp.* is *L. intracellularis.*

31. A polyclonal or monoclonal antibody molecule that is capable of binding specifically to a SodC polypeptide or a derivative of a SodC polypeptide that is derived from *Lawsonia spp.* and has at least about 70% sequence identity overall to the amino acid sequence set forth in SEQ ID NO: 1.

32. The antibody molecule of claim 31 wherein the SodC polypeptide or derivative thereof comprises the amino acid sequence set forth in SEQ ID NO: 1.

33. The antibody molecule of claim 31 wherein the SodC polypeptide or derivative thereof comprises about amino acid 1 to about amino acid 42 of SEQ ID NO: 1.

34. A method of diagnosing infection of a porcine or avian animal by *Lawsonia intracellularis* or a microorganism that is immunologically cross-reactive thereto, said method comprising the steps of contacting a biological sample derived from said animal with the antibody molecule of claim 31 for a time and under conditions sufficient

for an antigen:antibody complex to form, and then detecting said complex formation.

35. The method of claim 34 wherein the biological sample comprises whole serum, lymph nodes, ileum, caecum, small intestine, large intestine, faeces or a rectal swab derived from a porcine animal.

36. A method of identifying whether or not a porcine or avian animal has suffered from a past infection, or is currently infected, with *Lawsonia intracellularis* or a microorganism that is immunologically cross-reactive thereto, said method comprising contacting blood or serum derived from said animal with the immunogenic polypeptide of claim 1 for a time and under conditions sufficient for an antigen:antibody complex to form and then detecting said complex formation.

37. An isolated nucleic acid molecule which comprises a sequence of nucleotides that encodes, or is complementary to a nucleic acid molecule that encodes, a peptide, oligopeptide or polypeptide selected from the following:

(i) a peptide, oligopeptide or polypeptide which comprises an amino acid sequence which has at least about 70% sequence identity overall to the amino acid sequence set forth in SEQ ID NO: 1;

(ii) a peptide, oligopeptide or polypeptide which comprises an amino acid sequence which comprises an amino having at least about 50% sequence identity overall to amino acid residue 1 to about amino acid residue 42 of SEQ ID NO: 1; or

(iii) a homologue, analogue or derivative of (i) or (ii) which mimics a B-cell or T-cell epitope of *Lawsonia spp.*

38. The isolated nucleic acid molecule of claim 37, wherein the peptide, oligopeptide or polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 1 or about amino acid residue 1 to about amino acid residue 42 thereof, or the amino acid sequence encoded by the SodC-encoding nucleotide sequence of pALK14 (ATCC 207155), or a B-cell epitope or T-cell epitope thereof.

39. The isolated nucleic acid molecule of claim 38 comprising the nucleotide sequence set forth in SEQ ID NO: 2 or a complementary nucleotide sequence thereto, or a degenerate variant thereof.

40. The isolated nucleic acid molecule of claim 39 consisting essentially of the nucleotide sequence of SEQ ID NO: 2 or a degenerate variant thereof.

41. The isolated nucleic acid molecule of claim 38 comprising that portion of the nucleotide sequence of SEQ ID NO: 2, or a degenerate variant thereof, which encodes about amino acid residue 1 to about amino acid residue 42 of SEQ ID NO: 2.

42. The isolated nucleic acid molecule of claim 41 consisting essentially of that portion of the nucleotide sequence of SEQ ID NO: 2, or a degenerate variant thereof, which encodes about amino acid residue 1 to about amino acid residue 42 of SEQ ID NO: 2

43. A method of detecting *Lawsonia intracellularis* or related microorganism in a biological sample derived from a porcine or avian animal subject, said method comprising the steps of hybridising one or more probes or primers derived from the nucleotide sequence set forth in SEQ ID NO: 2 or a complementary nucleotide sequence thereto to said sample and then detecting said hybridisation using a detection means.

44. The method of claim 43 wherein the biological sample comprises whole serum, lymph nodes, ileum, caecum, small intestine, large intestine, faeces or a rectal swab derived from a porcine animal.

45. The method of claim 44 wherein the detection means comprises any nucleic acid based hybridisation or amplification reaction.

46. A probe or primer having at least about 15 contiguous nucleotides in length derived from SEQ ID NO: 2 or a complementary nucleotide sequence thereto.

47. A plasmid designated pALK14 (ATCC Accession No. 207155).

48. The combination vaccine according to claim 27 wherein the second immunogenic component comprises an antigenic *L. intracellularis* peptide, polypeptide or protein selected from the group consisting of OmpH, FlgE, hemolysin and autolysin.

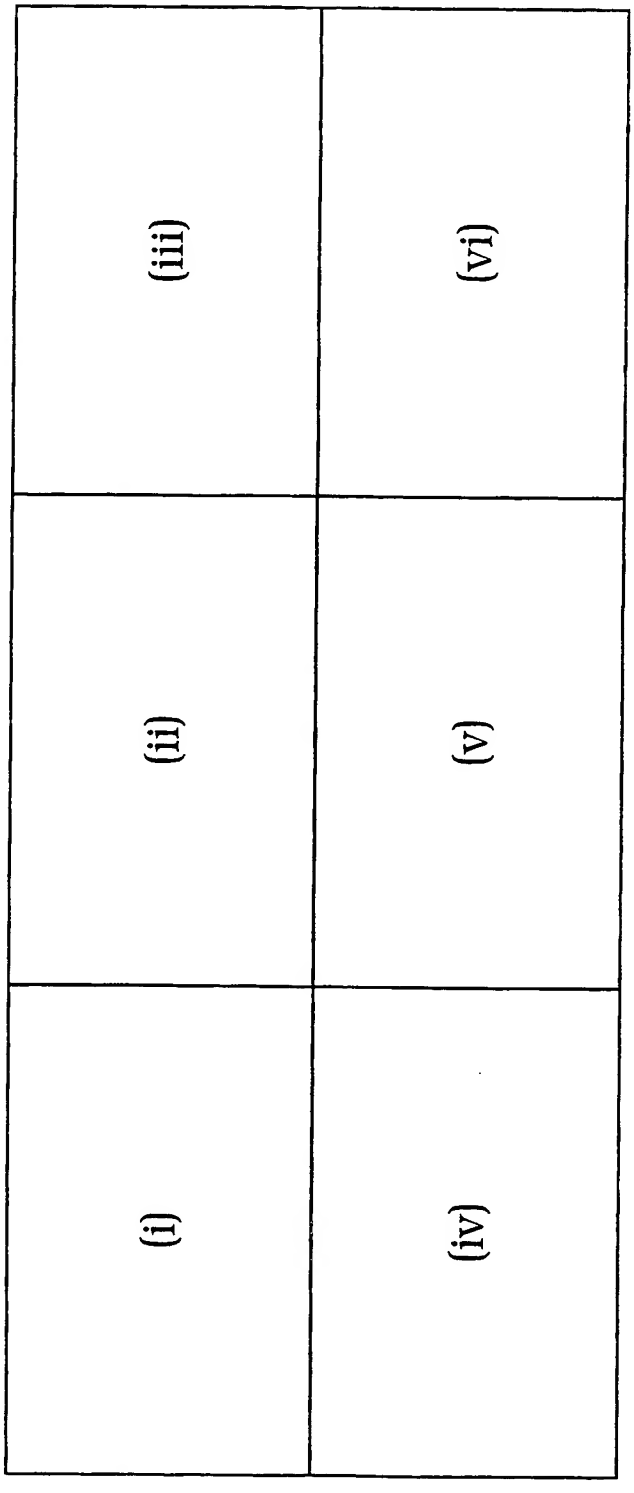

Figure 1

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S.	typhi	M	K	Y	T	I	L	S	L	V	A	G	A	L	I	S	C
P.	leio	M	N	K	A	K	T	L	L	F	T	A	L	A	F	G	L
H.	par	M	M	K	M	K	T	L	L	A	L	A	I	S	G	I	C
B.	abo	M	K	S	L	F	I	A	S	T	M	V	L	M	A	F	P
A.	pleur	M	K	L	T	N	L	A	L	A	F	T	L	F	G	A	S
L.	pneu	M	N	K	S	G	I	I	L	I	G	T	I	L	F	S	S

L.	int	-	-	-	-	-	-	-	-	-	-	C	S	V	T	S	E
E.	coli	-	-	-	-	-	-	-	-	-	-	-	-	A	S	E	K
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B.	abo	-	-	-	-	-	-	-	-	-	-	-	-	E	S	T	T
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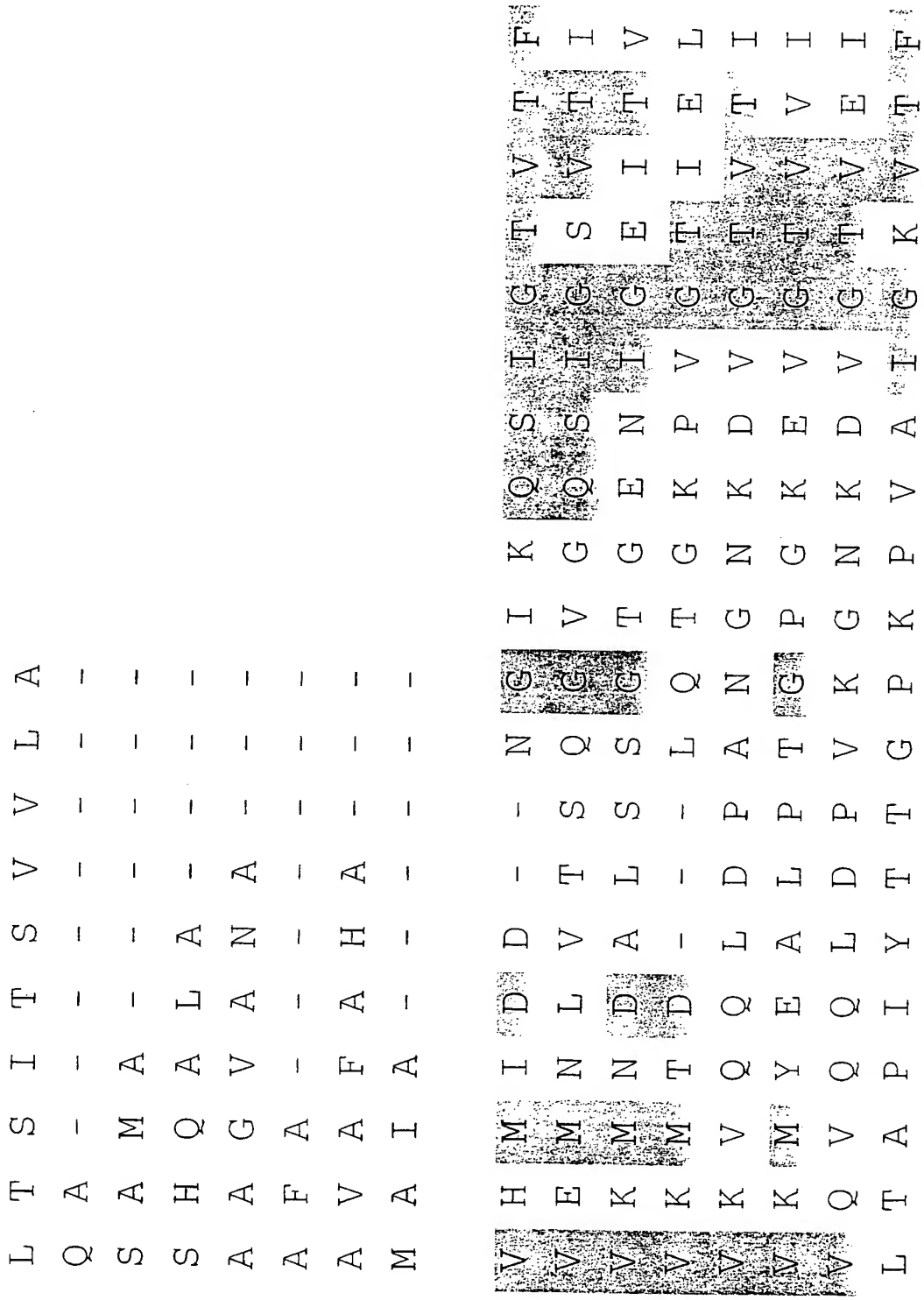
Figure 1(i)



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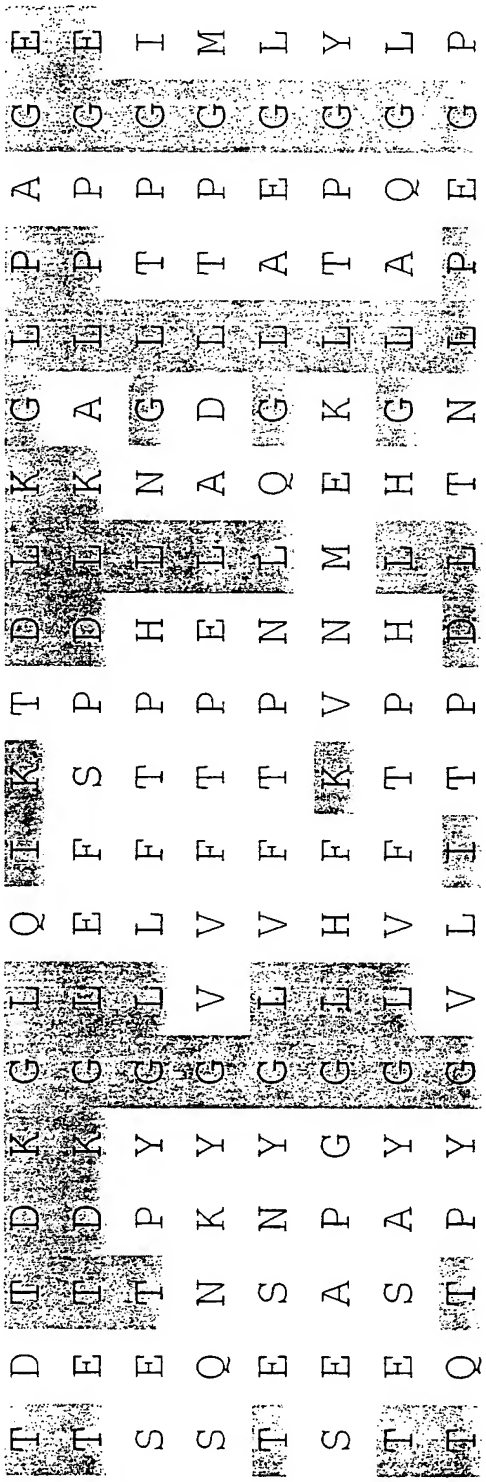

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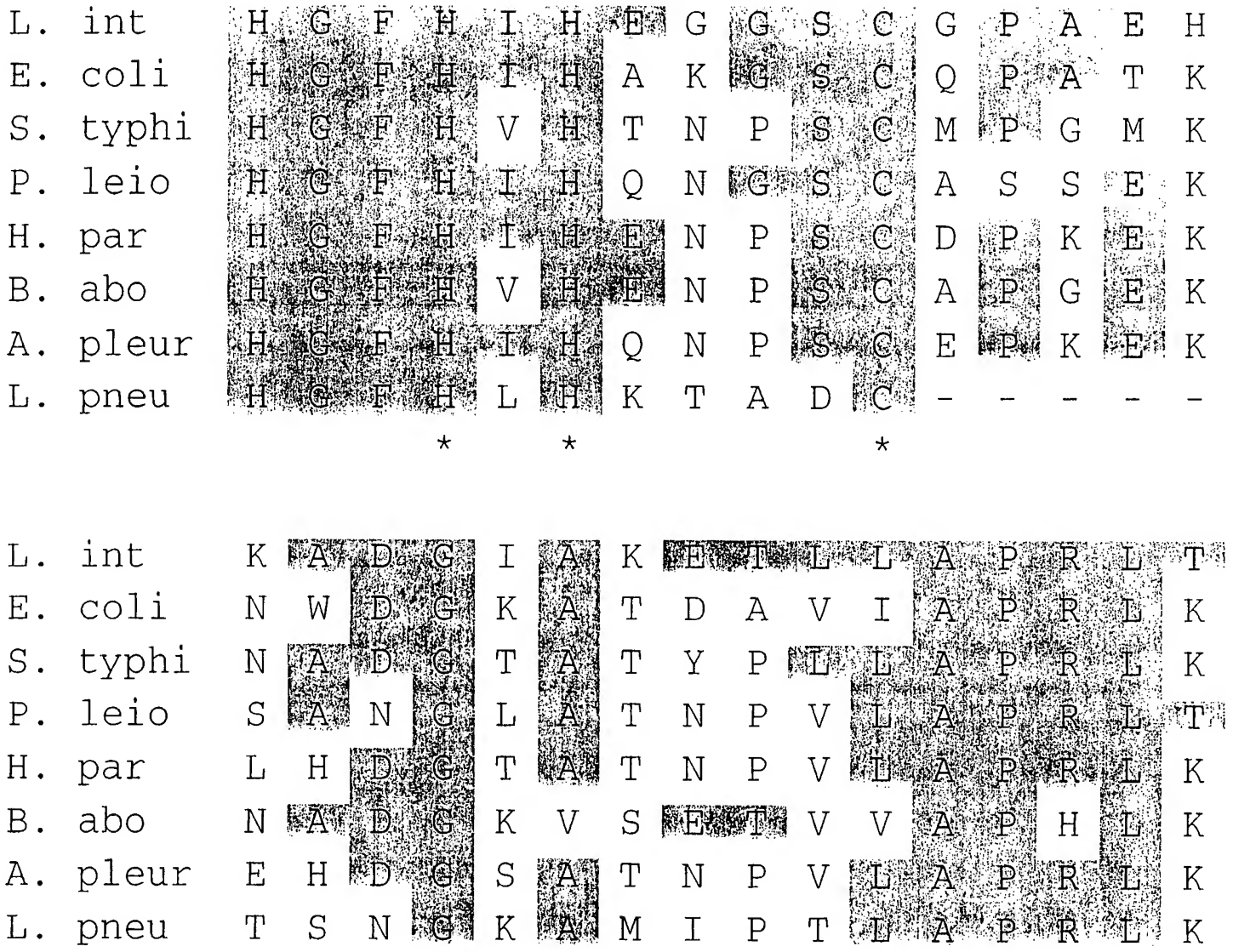
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P. leio H G F H I H Q N G S C A S S E K
H. par H G F H I H E N P S C D P K E K
B. abo H G F H V H E N P S C A P G E K
A. pleur H G F H I H Q N P S C E P K E K
L. pneu H G F H L H K T A D C - - - - -
* * *
L. int K A D G I A K E T L L A P R L T
E. coli N W D G K A T D A V I A P R L K
S. typhi N A D G T A T Y P L L A P R L K
P. leio S A N G L A T N P V L A P R L T
H. par L H D G T A T N P V L A P R L K
B. abo N A D G K V S E T V V A P H L K
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Figure 1(iv)

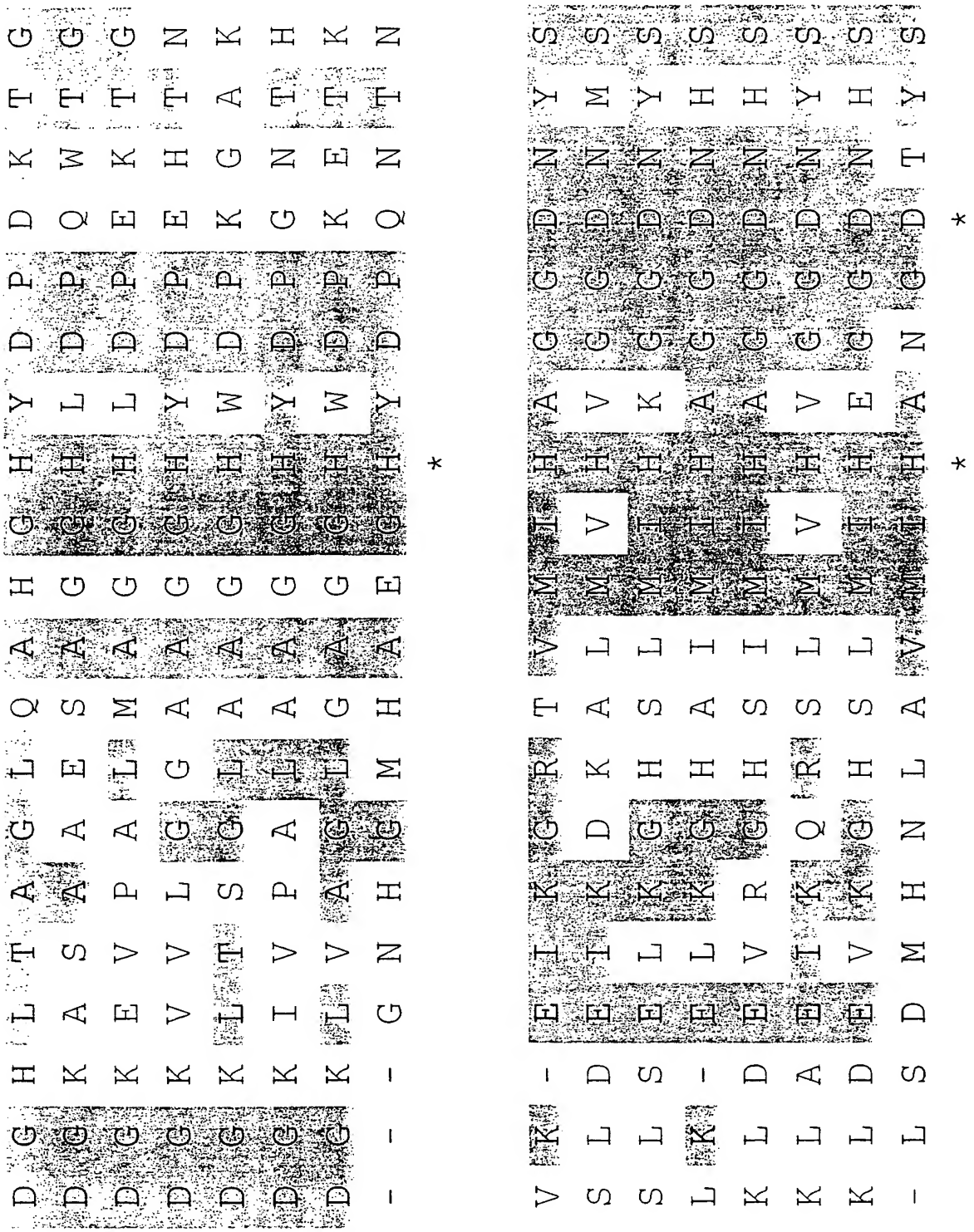

Figure 1(v)

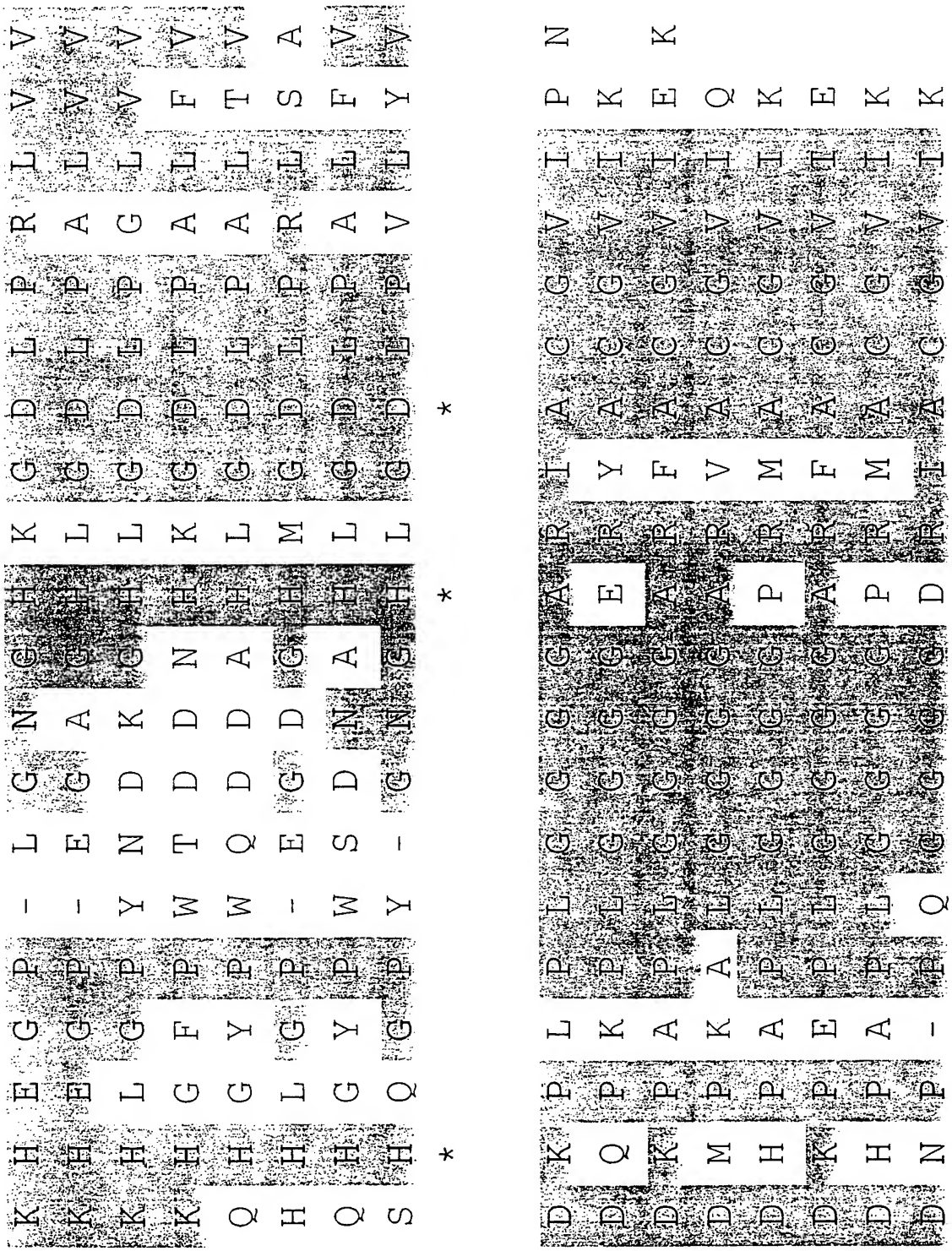

Figure 1(vi)

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